Davis, S.
09/189415



(FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, PHIC, PHIN, TOXCENTER' ENTERED AT 10:11:20 ON 17 DEC 2003)

- Author(s)

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L1          1363 SEA ABB=ON  PLU=ON  "FINLAY B"?/AU

L3            74 SEA ABB=ON  PLU=ON  ("DEVINNEY R"? OR "DE VINNEY R"?)/AU

L4          5120 SEA ABB=ON  PLU=ON  "STEIN M"?/AU

L5           373 SEA ABB=ON  PLU=ON  "KENNY B"?/AU
L6             7 SEA ABB=ON  PLU=ON  L1 AND L5 AND L3 AND L4
L7           118 SEA ABB=ON  PLU=ON  L1 AND (L5 OR L3 OR L4)
L8            28 SEA ABB=ON  PLU=ON  L5 AND (L3 OR L4)
L9            11 SEA ABB=ON  PLU=ON  L3 AND L4
L10          608 SEA ABB=ON  PLU=ON  (L7 OR L8 OR L1 OR L3 OR L4 OR L5)
                 AND RECEPTOR
L11          166 SEA ABB=ON  PLU=ON  L10 AND PATHOGEN?

L12           94 SEA ABB=ON  PLU=ON  (L7 OR L8 OR L1 OR L3 OR L4 OR L5)
                 AND RECEPTOR(S) HOST
L13           43 SEA ABB=ON  PLU=ON  L12 AND PATHOGEN?(S) BACTERI##
L14           48 SEA ABB=ON  PLU=ON  L6 OR L9 OR L13
L15           23 DUP REM L14 (25 DUPLICATES REMOVED)
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L15 ANSWER 1 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 1

ACCESSION NUMBER: 2003:770939 HCAPLUS

TITLE: **Bacterial pathogenesis:** exploiting cellular adherence

AUTHOR(S): Boyle, Erin C.; **Finlay, B. Brett**

CORPORATE SOURCE: Biotechnology Laboratory, Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Current Opinion in Cell Biology (2003), 15(5), 633-639
CODEN: COCBE3; ISSN: 0955-0674

PUBLISHER: Elsevier Science Ltd.

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review. Cell adhesion mols., such as integrins, cadherins, the Ig superfamily of cell adhesion mols. and selectins, play important structural roles and are involved in various signal transduction processes. As an initial step in the infectious process, many **bacterial pathogens** adhere to cell adhesion mols. as a means of exploiting the underlying signaling pathways, entering into host cells or establishing extracellular persistence. Often, bacteria are able to bind to cell adhesion mols. by mimicking or acting in place of **host** cell **receptors** or their ligands. Recent studies have contributed to the authors' understanding of bacterial adherence mechanisms and the consequences of receptor engagement; they have also highlighted alternative functions of cell adhesion mols.

REFERENCE COUNT: 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 2 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2003151440 EMBASE

TITLE: Citrobacter rodentium translocated intimin receptor

(Tir) is an essential virulence factor needed for actin condensation, intestinal colonization and colonic hyperplasia in mice.

AUTHOR: Deng W.; Vallance B.A.; Li Y.; Puente J.L.; **Finlay B.B.**

CORPORATE SOURCE: B.B. Finlay, Biotechnology Laboratory, University of British Columbia, Wesbrook Building, 6174 University Boulevard, Vancouver, BC V6T 1Z3, Canada. bfinlay@interchange.ubc.ca

SOURCE: Molecular Microbiology, (2003) 48/1 (95-115). Refs: 69
ISSN: 0950-382X CODEN: MOMIEE

COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
048 Gastroenterology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Citrobacter rodentium infection of mice serves as a relevant small animal model to study enterohaemorrhagic Escherichia coli (EHEC) and enteropathogenic E. coli (EPEC) infections in man. Enteropathogenic E. coli and EHEC translocate Tir into the **host** cytoplasmic membrane, where it serves as the **receptor** for the **bacterial** adhesin intimin and plays a central role in actin condensation beneath the adherent **bacterium**. In this report, we examined the function of C. rodentium Tir both in vitro and in vivo. Similar to EPEC, C. rodentium Tir is tyrosine phosphorylated and is essential for actin condensation. Citrobacter Tir and EPEC Tir are functionally interchangeable and both require tyrosine phosphorylation to mediate actin rearrangements. In contrast, Citrobacter Tir supports actin nucleation in EHEC independent of tyrosine phosphorylation, while EHEC Tir cannot replace Citrobacter Tir for this function. This indicates that C. rodentium and EPEC use an actin nucleating mechanism different from EHEC. We also found that Tir is expressed and translocated into mouse enterocytes in vivo by C. rodentium during infections. This represents the first direct demonstration of a type III effector translocated in vivo into a natural **host** by any **pathogen**. In addition, we showed that Tir, but not its tyrosine phosphorylation, is essential for C. rodentium to colonize the large bowel and induce attaching/effacing (A/E) lesions and colonic hyperplasia in mice, and that both EPEC Tir and EHEC Tir can substitute for Citrobacter Tir for these activities in vivo. These results thus demonstrate that Tir is an essential virulence factor in this infection model. The data also show that the function of Tir tyrosine phosphorylation and its subsequent actin nucleating activity are not essential for C. rodentium colonization of the mouse gut nor for inducing A/E lesions and colonic hyperplasia, thereby uncoupling colonization and disease from actin condensation for this A/E **pathogen**.

L15 ANSWER 3 OF 23 EMBASE  on STN

ACCESSION NUMBER: 2002374344 EMBASE

TITLE: Modulation of inducible nitric oxide synthase expression by the attaching and effacing **bacterial pathogen** Citrobacter rodentium in infected mice.

AUTHOR: Vallance B.A.; Deng W.; De Grado M.; Chan C.; Jacobson K.; **Finlay B.B.**

CORPORATE SOURCE: B.B. Finlay, Biotechnology Laboratory, Wesbrook Building, University of British Columbia, 6174 University Blvd., Vancouver, BC V6T 1Z3, Canada. bfinlay@interchange.ubc.ca

SOURCE: Infection and Immunity, (2002) 70/11 (6424-6435). Refs: 67
ISSN: 0019-9567 CODEN: INFIBR

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Citrobacter rodentium belongs to the attaching and effacing family of enteric **bacterial pathogens** that includes both enteropathogenic and enterohemorrhagic Escherichia coli. These **bacteria** infect their **hosts** by colonizing the intestinal mucosal surface and intimately attaching to underlying epithelial cells. The abilities of these **pathogens** to exploit the cytoskeleton and signaling pathways of **host** cells are well documented, but their interactions with the **host**'s antimicrobial defenses, such as inducible nitric oxide synthase (iNOS), are poorly understood. To address this issue, we infected mice with C. rodentium and found that iNOS mRNA expression in the colon significantly increased during infection. Immunostaining identified epithelial cells as the major source for immunoreactive iNOS. Finding that nitric oxide (NO) donors were bacteriostatic for C. rodentium in vitro, we examined whether iNOS expression contributed to **host** defense by infecting iNOS-deficient mice. Loss of iNOS expression caused a small but significant delay in **bacterial** clearance without affecting tissue pathology. Finally, immunofluorescence staining was used to determine if iNOS expression was localized to infected cells by staining for the C. rodentium virulence factor, translocated intimin **receptor** (Tir), as well as iNOS. Interestingly, while more than 85% of uninfected epithelial cells expressed iNOS, fewer than 15% of infected (Tir-positive) cells expressed detectable iNOS. These results demonstrate that both iNOS and intestinal epithelial cells play an active role in **host** defense during C. rodentium infection. However, the selective expression of iNOS by uninfected but not infected cells suggests that this **pathogen** has developed mechanisms to locally limit its exposure to **host**-derived NO.

L15 ANSWER 4 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2002183326 EMBASE

TITLE: Co-ordinate regulation of distinct host cell signalling pathways by multifunctional enteropathogenic Escherichia coli effector molecules.

AUTHOR: **Kenny B.**; Ellis S.; Leard A.D.; Warawa J.; Mellor H.; Jepson M.A.

CORPORATE SOURCE: B. Kenny, Department of Pathology, School of Medical Sciences, University Walk, Bristol BS8 1TD, United Kingdom. B.Kenny@bristol.ac.uk

SOURCE: Molecular Microbiology, (2002) 44/4 (1095-1107).

Refs: 50
ISSN: 0950-382X CODEN: MOMIEE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Enteropathogenic Escherichia coli (EPEC) is a major cause of paediatric diarrhoea and a model for the family of attaching and effacing (A/E) **pathogens**. A/E **pathogens** encode a type III secretion system to transfer effector proteins into **host** cells. The EPEC Tir effector protein acts as a **receptor** for the **bacterial** surface protein intimin and is involved in the formation of Cdc42-independent, actin-rich pedestal structures beneath the adhered **bacteria**. In this paper, we demonstrate that EPEC binding to HeLa cells also induces Tir-independent, cytoskeletal rearrangement evidenced by the early, transient formation of filopodia-like structures at sites of infection. Filopodia formation is dependent on expression of the EPEC Map effector molecule - a protein that targets mitochondria and induces their dysfunction. We show that Map-induced filopodia formation is independent of mitochondrial targeting and is abolished by cellular expression of the Cdc42 inhibitory WASP-CRIB domain, demonstrating that Map has at least two distinct functions in **host** cells. The transient nature of the filopodia is related to an ability of EPEC to downregulate Map-induced cell signalling that, like pedestal formation, was dependent on both Tir and intimin proteins. The ability of Tir to downregulate filopodia was impaired by disrupting a putative GTPase-activating protein (GAP) motif, suggesting that Tir may possess such a function, with its interaction with intimin triggering this activity. Furthermore, we also found that Map-induced cell signalling inhibits pedestal formation, revealing that the cellular effects of Tir and Map must be co-ordinately regulated during infection. Possible implications of the multifunctional nature of EPEC effector molecules in **pathogenesis** are discussed.

L15 ANSWER 5 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 2

ACCESSION NUMBER: 2002:274824 HCAPLUS
DOCUMENT NUMBER: 137:167018
TITLE: Mechanism of action of EPEC type III effector molecules
AUTHOR(S): **Kenny, Brendan**
CORPORATE SOURCE: Department of Pathology and Microbiology, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK
SOURCE: International Journal of Medical Microbiology (2002), 291(6-7), 469-477
CODEN: IMEMFV; ISSN: 1438-4221
PUBLISHER: Urban & Fischer Verlag GmbH & Co. KG
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review. Enteropathogenic E. coli (EPEC) is a prototypic member of the family of related "attaching and effacing (A/E)" pathogens that induce diarrheal disease, especially to the young that can be fatal, of a wide range of mammalian species. Disease is correlated with the loss of absorptive gut epithelial microvilli and the reorganization of host cytoskeletal proteins into pedestal-like structures beneath

the adherent bacteria. These phenotypes are dependent on a **pathogenicity** island (LEE; Locus of Enterocyte Effacement) encoding a type III secretion system, secreted proteins, chaperone mols., regulatory proteins and the **bacterial** outer membrane protein intimin. The type III secretion apparatus directs the transfer of specific proteins across the bacterial envelope, with a subset (EPEC secreted proteins - EspA, EspB and EspD) functioning to transfer effector proteins into host cells. These effector mols. subvert cellular processes that undoubtedly benefit the pathogen and contribute to disease. Three LEE-encoded EPEC effector mols. have so far been identified with one, Tir (Translocated intimin **receptor**), being transferred into **host** cells where it is modified by **host** kinases and becomes inserted into the plasma membrane to orchestrate cytoskeletal rearrangements linked to disease. This activity is dependent on its interaction with intimin and on tyrosine phosphorylation, with Tir-intimin interaction essential for virulence. A second effector Map, Mitochondrial-associated protein, is targeted to mitochondria where it has membrane-potential disrupting activity. The third, EspF disrupts intestinal barrier function and can induce host cell death by unknown mechanisms. Recent data relating to the mechanism by which Tir and Map function within host cells is discussed.

REFERENCE COUNT: 63 THERE ARE 63 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 6 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2002144140 EMBASE
TITLE: Bacterial avoidance of phagocytosis.
AUTHOR: Celli J.; **Finlay B.B.**
CORPORATE SOURCE: J. Celli, Biotechnology Laboratory, University of British Columbia, 6174 University Boulevard, Vancouver, BC V6T 1Z3, Canada. bfinlay@interchange.ubc.ca
SOURCE: Trends in Microbiology, (1 May 2002) 10/5 (232-237). Refs: 45
ISSN: 0966-842X CODEN: TRMIEA
PUBLISHER IDENT.: S 0966-842X(02)02343-0
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Phagocytosis constitutes the primary line of **host** innate and adaptive defence against incoming microbial **pathogens**, providing an efficient means for their removal and destruction. However, several virulent **bacteria** that do not function as intracellular **pathogens** have evolved mechanisms to avoid and prevent phagocytosis that constitute an essential part of their **pathogenic** capacity. Some of these mechanisms include preventing recognition by phagocytic **receptors** or blocking uptake by professional phagocytes. Recently, the molecular mechanisms of such antiphagocytic properties have been elucidated for some **pathogens**. Such mechanisms illustrate the diversity of mechanisms **bacterial pathogens** use to avoid phagocytic uptake.

L15 ANSWER 7 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 2001:761778 HCAPLUS
DOCUMENT NUMBER: 136:66672
TITLE: Enterohemorrhagic and enteropathogenic Escherichia coli use a different Tir-based mechanism for pedestal formation
AUTHOR(S): **DeVinney, Rebekah**; Puente, Jose Luis; Gauthier, Annick; Goosney, Danika; **Finlay, B. Brett**
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Molecular Microbiology (2001), 41(6), 1445-1458
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Enterohemorrhagic Escherichia coli (EHEC) adheres to the host intestinal epithelium, resulting in the formation of actin pedestals beneath adhering bacteria. EHEC and a related **pathogen**, enteropathogenic Escherichia coli (EPEC), insert a **bacterial receptor**, Tir, into the **host** plasma membrane, which is required for pedestal formation. An important difference between EPEC and EHEC Tir is that EPEC but not EHEC Tir is tyrosine phosphorylated once delivered into the host. In this study, we assessed the role of Tir tyrosine phosphorylation in pedestal formation by EPEC and EHEC. In EPEC, pedestal formation is absolutely dependent on Tir tyrosine phosphorylation and is not complemented by EHEC Tir. The protein sequence surrounding EPEC Tir tyrosine 474 is critical for Tir tyrosine phosphorylation and pedestal formation by EPEC. In contrast, Tir tyrosine phosphorylation is not required for pedestal formation by EHEC. EHEC forms pedestals with both wild-type EPEC Tir and the nontyrosine-phosphorylatable EPEC Tir Y474F. Pedestal formation by EHEC requires the type III delivery of addnl. EHEC factors into the host cell. These findings highlight differences in the mechanisms of pedestal formation by these closely related pathogens and indicate that EPEC and EHEC modulate different signaling pathways to affect the host actin cytoskeleton.
REFERENCE COUNT: 59 THERE ARE 59 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 8 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2001113008 EMBASE
TITLE: Enteropathogenic Escherichia coli mediates antiphagocytosis through the inhibition of PI 3-kinase-dependent pathways.
AUTHOR: Celli J.; Olivier M.; **Finlay B.B.**
CORPORATE SOURCE: B.B. Finlay, Biotechnology Laboratory, University of British Columbia, Vancouver, BC V6T 1Z3, Canada. bfinlay@interchange.ubc.ca
SOURCE: EMBO Journal, (15 Mar 2001) 20/6 (1245-1258).
Refs: 56
ISSN: 0261-4189 CODEN: EMJODG
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology

LANGUAGE: English
SUMMARY LANGUAGE: English
AB The extracellular **pathogen** enteropathogenic Escherichia coli (EPEC) uses a type III secretion system to inhibit its uptake by macrophages. We show that EPEC antiphagocytosis is independent of the translocated intimin **receptor** Tir and occurs by preventing F-actin polymerization required for **bacterial** uptake. EPEC-macrophage contact triggered activation of phosphatidylinositol (PI) 3-kinase, which was subsequently inhibited in a type III secretion-dependent manner. Inhibition of PI 3-kinase significantly reduced uptake of a secretion-deficient mutant, without affecting antiphagocytosis by the wild type, suggesting that EPEC blocks a PI 3-kinase-dependent phagocytic pathway. EPEC specifically inhibited Fcγ **receptor**- but not CR3-**receptor** mediated phagocytosis of opsonized zymosan. We showed that EPEC inhibits PI 3-kinase activity rather than its recruitment to the site of **bacterial** contact. Phagocytosis of a secretion mutant correlated with the association of PI 3-kinase with tyrosine-phosphorylated proteins, which wild-type EPEC prevented. These results show that EPEC blocks its uptake by inhibiting a PI 3-kinase-mediated pathway, and translocates effectors other than Tir to interfere with actin-driven **host** cell processes. This constitutes a novel mechanism of phagocytosis avoidance by an extracellular **pathogen**.

L15 ANSWER 9 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 2001:699619 HCAPLUS
DOCUMENT NUMBER: 136:3899
TITLE: Enteropathogenic E. coli Tir binds Nck to initiate actin pedestal formation in host cells
AUTHOR(S): Gruenheid, Samantha; **DeVinney, Rebekah**; Bladt, Friedhelm; Goosney, Danika; Gelkop, Sigal; Gish, Gerald D.; Pawson, Tony; **Finlay, B. Brett**
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1G3, Can.
SOURCE: Nature Cell Biology (2001), 3(9), 856-859
CODEN: NCBIFN; ISSN: 1465-7392
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Enteropathogenic Escherichia coli (EPEC) is a **bacterial** **pathogen** that causes infantile diarrhea worldwide. EPEC injects a bacterial protein, translocated intimin **receptor** (Tir), into the **host**-cell plasma membrane where it acts as a **receptor** for the bacterial outer membrane protein, intimin. The interaction of Tir and intimin triggers a marked rearrangement of the host actin cytoskeleton into pedestals beneath adherent bacteria. On delivery into host cells, EPEC Tir is phosphorylated on tyrosine 474 of the intracellular carboxy-terminal domain, an event that is required for pedestal formation. Despite its essential role, the function of Tir tyrosine phosphorylation has not yet been elucidated. Here we show that tyrosine 474 of Tir directly binds the host-cell adaptor protein Nck, and that Nck is required for the recruitment of both neural Wiskott-Aldrich-syndrome protein (N-WASP) and the actin-related protein (Arp)2/3 complex to the EPEC pedestal, directly linking Tir to the cytoskeleton. Cells with null alleles of both mammalian Nck genes are resistant to the

effects of EPEC on the actin cytoskeleton. These results implicate Nck adaptors as host-cell determinants of EPEC virulence.
REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 10 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2000287269 EMBASE
TITLE: Exploitation of host cells by enteropathogenic Escherichia coli.
AUTHOR: Vallance B.A.; **Finlay B.B.**
CORPORATE SOURCE: B.B. Finlay, Biotechnology Laboratory, Wesbrook Building, University of British Columbia, 6174 University Boulevard, Vancouver, BC V6T 1Z3, Canada. bfinlay@interchange.ubc.ca
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1 Aug 2000) 97/16 (8799-8806).
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Microbial **pathogens** have evolved many ingenious ways to infect their **hosts** and cause disease, including the subversion and exploitation of target **host** cells. One such subversive microbe is enteropathogenic Escherichia coli (EPEC). A major cause of infantile diarrhea in developing countries, EPEC poses a significant health threat to children worldwide. Central to EPEC-mediated disease is its colonization of the intestinal epithelium. After initial adherence, EPEC causes the localized effacement of microvilli and intimately attaches to the **host** cell surface, forming characteristic attaching and effacing (A/E) lesions. Considered the prototype for a family of A/E lesion-causing **bacteria**, recent in vitro studies of EPEC have revolutionized our understanding of how these **pathogens** infect their **hosts** and cause disease. Intimate attachment requires the type III-mediated secretion of **bacterial** proteins, several of which are translocated directly into the infected cell, including the **bacteria**'s own **receptor** (Tir). Binding to this membrane-bound, **pathogen**-derived protein permits EPEC to intimately attach to mammalian cells. The translocated EPEC proteins also activate signaling pathways within the underlying cell, causing the reorganization of the **host** actin cytoskeleton and the formation of pedestal-like structures beneath the adherent **bacteria**. This review explores what is known about EPEC's subversion of mammalian cell functions and how this knowledge has provided novel insights into **bacterial** **pathogenesis** and microbe-**host** interactions. Future studies of A/E **pathogens** in animal models should provide further insights into how EPEC exploits not only epithelial cells but other **host** cells, including those of the immune system, to cause diarrheal disease.

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RESERVED. on STN
ACCESSION NUMBER: 2000245593 EMBASE
TITLE: Crystal structure of enteropathogenic Escherichia coli intimin-receptor complex.
AUTHOR: Luo Y.; Frey E.A.; Pfuetzner R.A.; Creagh A.L.; Knoechel D.G.; Haynes C.A.; **Finlay B.B.**; Strynadka N.C.J.
CORPORATE SOURCE: N.C.J. Strynadka, Dept. of Biochem. and Molec. Biology, Biotechnology Laboratory, University of British Columbia, Vancouver, BC V6T 1Z3, Canada
SOURCE: Nature, (29 Jun 2000) 405/6790 (1073-1077). ISSN: 0028-0836 CODEN: NATUAS
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Intimin and its translocated intimin **receptor** (Tir) are **bacterial** proteins that mediate adhesion between mammalian cells and attaching and effacing (ME) **pathogens**. Enteropathogenic Escherichia coli (EPEC) causes significant paediatric morbidity and mortality world-wide. A related ME **pathogen**, enterohaemorrhagic E. coli (EHEC; O157:H7) is one of the most important food-borne **pathogens** in North America, Europe and Japan. A unique and essential feature of A/E **bacterial pathogens** is the formation of actin-rich pedestals beneath the intimately adherent **bacteria** and localized destruction of the intestinal brush border. The **bacterial** outer membrane adhesin, intimin, is necessary for and diarrhoea. The ME **bacteria** the production of the ME lesion translocate their own **receptor** for intimin, Tir, into the membrane of mammalian cells using the type III secretion system. The translocated Tir triggers additional **host** signalling events and actin nucleation, which are essential for lesion formation. Here we describe the crystal structures of an EPEC intimin carboxy-terminal fragment alone and in complex with the EPEC Tir intimin-binding domain, giving insight into the molecular mechanisms of adhesion of ME **pathogens**.

L15 ANSWER 12 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2001013073 EMBASE
TITLE: Gut feelings: Enteropathogenic E. coli (EPEC) interactions with the host.
AUTHOR: Goosney D.L.; Gruenheid S.; **Finlay B.B.**
CORPORATE SOURCE: D.L. Goosney, Biotechnology Laboratory, University of British Columbia, Vancouver, BC, Canada. bfinlay@interchange.ubc.ca
SOURCE: Annual Review of Cell and Developmental Biology, (2000) 16/- (173-189). Refs: 83 ISSN: 1081-0706 CODEN: ARDBF8
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Enteropathogenic Escherichia coli (EPEC) is a gram-negative

**bacterial pathogen** that adheres to human intestinal epithelial cells, resulting in watery, persistent diarrhea. It subverts the **host** cell cytoskeleton, causing a rearrangement of cytoskeletal components into a characteristic pedestal structure underneath adherent **bacteria**. In contrast to other intracellular **pathogens** that affect the actin cytoskeleton from inside the **host** cytoplasm, EPEC remains extracellular and transmits signals through the **host** cell plasma membrane via direct injection of virulence factors by a "molecular syringe" the **bacterial** type III secretion system. One injected factor is Tir, which functions as the plasma membrane **receptor** for EPEC adherence. Tir directly links extracellular EPEC through the epithelial membrane and firmly anchors it to the **host** cell actin cytoskeleton, thereby initiating pedestal formation. In addition to stimulating actin nucleation and polymerization in the **host** cell, EPEC activates several other signaling pathways that lead to tight junction disruption, inhibition of phagocytosis, altered ion secretion, and immune responses. This review summarizes recent developments in our understanding of EPEC **pathogenesis** and discusses similarities and differences between EPEC pedestals, focal contacts, and Listeria monocytogenes actin tails.

L15 ANSWER 13 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 5
ACCESSION NUMBER: 2000:240248 HCAPLUS
DOCUMENT NUMBER: 133:15368
TITLE: Enteropathogenic Escherichia coli (EPEC) attachment to epithelial cells: exploiting the host cell cytoskeleton from the outside
AUTHOR(S): Celli, Jean; Deng, Wanyin; **Finlay, B. Brett**
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Cellular Microbiology (2000), 2(1), 1-9
CODEN: CEMIF5; ISSN: 1462-5814
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review, with 54 refs. Enteropathogenic Escherichia coli (EPEC), a leading cause of human infantile diarrhea, is the prototype for a family of intestinal **bacterial pathogens** that induce attaching and effacing (A/E) lesions on host cells. A/E lesions are characterized by localized effacement of the brush border of enterocytes, intimate bacterial attachment and pedestal formation beneath the adherent bacteria. As a result of some recent breakthrough discoveries, EPEC has now emerged as a fascinating paradigm for the study of host-pathogen interactions and cytoskeletal rearrangements that occur at the host cell membrane. EPEC uses a type III secretion machinery to attach to epithelial cells, translocating its own **receptor** for intimate attachment, Tir, into the **host** cell, which then binds to intimin on the bacterial surface. Studies of EPEC-induced cytoskeletal rearrangements have begun to provide clues as to the mechanisms used by this pathogen to subvert the host cell cytoskeleton and signaling pathways. These findings have unraveled new ways by which **pathogenic bacteria** exploit host processes from the cell surface and have shed new light on how EPEC might cause diarrhea.

REFERENCE COUNT: 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 14 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 6
ACCESSION NUMBER: 1999:326051 HCAPLUS
DOCUMENT NUMBER: 130:333761
TITLE: Pathogenic Escherichia coli intimin receptor Tir and gene tir and methods for detecting gene tir or Tir protein and for drug screening
INVENTOR(S): **Finlay, B. Brett; Kenny, Brendan; Devinney, Rebekah; Stein, Marcus**
PATENT ASSIGNEE(S): University of British Columbia, Can.
SOURCE: PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9924576 | A1 | 19990520 | WO 1998-CA1042 | 19981110 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2309559 | AA | 19990520 | CA 1998-2309559 | 19981110 |
| AU 9911373 | A1 | 19990531 | AU 1999-11373 | 19981110 |
| EP 1029054 | A1 | 20000823 | EP 1998-954076 | 19981110 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| JP 2001522605 | T2 | 20011120 | JP 2000-520570 | 19981110 |

PRIORITY APPLN. INFO.: US 1997-65130P P 19971112
WO 1998-CA1042 W 19981110

AB A polypeptide, called Tir (for translocated intimin receptor), which is secreted by attaching and effacing pathogens, such as the enteropathogenic (EPEC) and enterohemorrhagic (EHEC) E. coli is disclosed. These **bacterial pathogens** insert their own **receptors** into mammalian cell surfaces, to which the **bacterial pathogen** then adheres to trigger addnl. **host** signaling events and actin nucleation. Diagnosis of disease caused by pathogenic E. coli can be performed by the use of antibodies which bind to Tir to detect the protein or the use of nucleic acid probes for detection of nucleic acids encoding Tir polypeptide. Isolated nucleic acid sequences encoding Tir polypeptide, Tir peptides, a recombinant method for producing recombinant Tir, antibodies which bind to Tir, and a kit for the detection of Tir-producing E. coli are provided. A method of immunizing a host with Tir to induce a protective immune response to Tir or a second polypeptide of interest is also provided. A method for screening for compds. which interfere with the binding of

**bacterial pathogens** to their receptors is further provided. Thus, protein Hp90, previously believed to be a **host** membrane protein, has been identified as an EHEC- or EPEC-secreted protein which acts as an intimin **receptor**. Proteins encoded by the espA and espB genes were necessary for delivery of Tir to the host membrane.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 15 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 7
ACCESSION NUMBER: 1999:291203 HCAPLUS
DOCUMENT NUMBER: 131:85390
TITLE: Enterohemorrhagic Escherichia coli O157:H7 produces Tir, which is translocated to the host cell membrane but is not tyrosine phosphorylated
AUTHOR(S): **DeVinney, Rebekah; Stein, Markus**; Reinscheid, Dieter; Abe, Akio; Ruschkowski, Sharon; Finlay, B. Brett
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1ZA, Can.
SOURCE: Infection and Immunity (1999), 67(5), 2389-2398
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Intimate attachment to the host cell leading to the formation of attaching and effacing (A/E) lesions is an essential feature of enterohemorrhagic Escherichia coli (EHEC) O157:H7 pathogenesis. In a related pathogen, enteropathogenic E. coli (EPEC), this activity is dependent upon translocation of the intimin receptor, Tir, which becomes tyrosine phosphorylated within the host cell membrane. In contrast, the accumulation of tyrosine-phosphorylated proteins beneath adherent EHEC bacteria does not occur, leading to questions about whether EHEC uses a Tir-based mechanism for adherence and A/E lesion formation. In this report, we demonstrate that EHEC produces a functional Tir that is inserted into host cell membranes, where it serves as an intimin receptor. However, unlike in EPEC, in EHEC Tir is not tyrosine phosphorylated yet plays a key role in both bacterial adherence to epithelial cells and pedestal formation. EHEC, but not EPEC, was unable to synthesize Tir in Luria-Bertani medium but was able to secrete Tir into M9 medium, suggesting that Tir synthesis and secretion may be regulated differently in these two pathogens. EHEC Tir and EPEC Tir both bind intimin and focus cytoskeletal rearrangements, indicating that tyrosine phosphorylation is not needed for pedestal formation. EHEC and EPEC intimins are functionally interchangeable, but EHEC Tir shows a much greater affinity for EHEC intimin than for EPEC intimin. These findings highlight some of the differences and similarities between EHEC and EPEC virulence mechanisms, which can be exploited to further define the mol. basis of pedestal formation.

REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 16 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1999064039 EMBASE

TITLE: Phosphorylation of tyrosine 474 of the enteropathogenic Escherichia coli (EPEC) Tir **receptor** molecule is essential for actin nucleating activity and is preceded by additional **host** modifications.

AUTHOR: **Kenny B.**
CORPORATE SOURCE: B. Kenny, Department of Pathology Microbiology, School of Medical Sciences, University Walk, Bristol BS8 1TD, United Kingdom. B.Kenny@bristol.ac.uk

SOURCE: Molecular Microbiology, (1999) 31/4 (1229-1241).
Refs: 33
ISSN: 0950-382X CODEN: MOMIEE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
005 General Pathology and Pathological Anatomy
029 Clinical Biochemistry
048 Gastroenterology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The enteropathogenic Escherichia coli (EPEC) Tir protein becomes tyrosine phosphorylated in host cells and displays an increase in apparent molecular mass. The interaction of Tir with the EPEC outer membrane protein, intimin, triggers actin nucleation beneath the adherent **bacteria**. The enterohaemorrhagic E. coli 0157:H7 (EHEC) Tir molecule is not tyrosine phosphorylated. In this paper, Tir tyrosine phosphorylation is shown to be essential for actin nucleation activity, but not for the increase in apparent molecular mass observed in target cells. Tyrosine phosphorylation had no role in Tir molecular mass shift, indicating additional host modifications. Analysis of Tir intermediates indicates that tyrosine-independent modification functions to direct Tir's correct insertion from the cytoplasm into the host membrane. Deletion analysis identified Tir domains participating in translocation, association with the host membrane, modification and antibody recognition. Intimin was found to bind a 55-amino-acid region (TIBA) within Tir that topological and sequence analysis suggests is located in an extracellular loop, Homologous TIBA sequences exist in integrins, which also bind intimin. Collectively, this study provides definitive evidence for the importance of tyrosine phosphorylation for EPEC Tir function and reveals differences in the **pathogenicity** of EPEC and EHEC. The data also suggest a mechanism for Tir insertion into the host membrane, as well as providing clues to the mode of intimin-integrin interaction.

L15 ANSWER 17 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 8
ACCESSION NUMBER: 1999:422677 HCAPLUS
TITLE: Enteropathogenic Escherichia coli. A pathogen that inserts its own **receptor** into **host** cells
AUTHOR(S): **De Vinney, R.**; Gauthier, A.; Abe, A.; **Finlay, B. B.**
CORPORATE SOURCE: Biotechnology Laboratory, Univ. British Columbia, Vancouver, BC, V6T 1Z4, Can.
SOURCE: Cellular and Molecular Life Sciences (1999), 55(6/7), 961-976
CODEN: CMLSFI; ISSN: 1420-682X
PUBLISHER: Birkhaeuser Verlag

DOCUMENT TYPE: Journal
LANGUAGE: English
AB Enteropathogenic Escherichia coli (EPEC) is a major cause of infant diarrhea, killing hundreds of thousands of children per yr worldwide. Intimate attachment to the host cell leading to the formation of actin-rich pedestals beneath the adhering **bacteria** is an essential feature of EPEC **pathogenesis**. EPEC attaches to host cells via the outer membrane adhesin, intimin. It was recently shown that EPEC inserts its own **receptor** for intimate adherence, Tir (translocated intimin **receptor**) into the **host** cell membrane. The focus of this review is on the discovery and characterization of this novel receptor, and our current understanding of its role in pedestal formation. Gram-neg. bacterial secretion systems, including type III secretion systems, are reviewed and discussed in the context of Tir delivery into the host cell membrane. The relationship and relevance of in vitro models compared to the actual in viva situation is essential to understanding disease. We have critically reviewed the use of animal models in studying EPEC infection. Elucidating the function of Tir will contribute to our understanding of how EPEC mediates disease.
REFERENCE COUNT: 122 THERE ARE 122 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 18 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1998187833 EMBASE
TITLE: EPEC delivers the goods.
AUTHOR: Kaper J.B.; **Finlay B.B.**; **DeVinney R.**; **Kenny B.**; **Stein M.**
CORPORATE SOURCE: J.B. Kaper, Center for Vaccine Development, University of Maryland, School of Medicine, 685 West Baltimore St, Baltimore, MD 21201, United States. jkaper@umaryland.edu
SOURCE: Trends in Microbiology, (1998) 6/5 (169-172).
Refs: 20
ISSN: 0966-842X CODEN: TRMIEA
PUBLISHER IDENT.: S 0966-842X(98)01266-9
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Note
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
048 Gastroenterology
LANGUAGE: English

L15 ANSWER 19 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1998348098 EMBASE
TITLE: Isolation and characterization of Salmonella typhimurium and Yersinia pseudotuberculosis-containing phagosomes from infected mouse macrophages: Y. pseudotuberculosis traffics to terminal lysosomes where they are degraded.
AUTHOR: Mills S.D.; **Finlay B.B.**
CORPORATE SOURCE: Prof. B.B. Finlay, Biotechnology Laboratory, Wesbrook Building, 6174 University Boulevard, Vancouver, BC V6T 1Z3, Canada. bfinlay@unixg.ubc.ca

SOURCE: European Journal of Cell Biology, (1998) 77/1 (35-47).
Refs: 43
ISSN: 0171-9335 CODEN: EJCBDN
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The interaction of Salmonella and Yersinia with macrophages is critical to the **pathogenesis** of these organisms. After internalization into macrophages, these **bacteria** reside in membrane-enclosed vacuoles. In this report, we present an approach to isolate and characterize **bacteria** containing vacuoles (BCVs) to study intracellular trafficking of **pathogenic bacteria** within the membrane system of **host** cells. Using the mouse monocyte-macrophage cell line J774A.1, we found that Salmonella typhimurium replicated intracellularly to approximately 5 times its original numbers over a 9 hour infection course, while Yersinia pseudotuberculosis and Escherichia coli did not replicate inside these cells. Analysis of isolated latex bead-containing vacuoles confirmed that they trafficked normally from endosomes to lysosomes within the endocytic pathway of J774A.1 cells. We isolated BCVs free of contaminating endosomes and lysosomes using sucrose step gradients, and used quantitative immunoblotting to characterize the contents of these vacuoles at different time points after internalization. We found that the isolated BCVs contained endosomal and lysosomal marker proteins including lamp-1, mannose 6-phosphate **receptor** (M 6-PR), cathepsin D and cathepsin L. Further, we report on differential processing of lysosomal hydrolases (such as cathepsin D and cathepsin L) associated with the isolated BCVs. Although there was some contamination of the S. typhimurium-containing vacuoles with endoplasmic reticulum (ER) marker protein calnexin, the Y. pseudotuberculosis-containing vacuoles were predominately free of ER contamination. The Y. pseudotuberculosis-containing vacuoles displayed properties of lysosomes, containing the M 6-PR-dependent lysosomal hydrolases cathepsin D and cathepsin L, which were shown to be processed to their mature forms incrementally over time. These results, coupled with intracellular growth and microscopic examination of infected cells over time, indicated that Y. pseudotuberculosis traffics to lysosomes where they are degraded. The described method for isolation and characterization of BCVs proved to be a valuable tool to characterize the vacuolar compartment occupied by Y. pseudotuberculosis, and has potential to be applied to other vacuole resident **pathogens** whose trafficking is thought to play a role in **pathogenesis**.

L15 ANSWER 20 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 9

ACCESSION NUMBER: 1997:755652 HCAPLUS
DOCUMENT NUMBER: 128:72707

TITLE: Enteropathogenic E. coli (EPEC) transfers its receptor for intimate adherence into mammalian cells

AUTHOR(S): **Kenny, Brendan; DeVinney, Rebekah; Stein, Markus;** Reinscheid, Dieter J.; Frey, Elizabeth A.; **Finlay, B. Brett**

CORPORATE SOURCE: Biotechnol. Lab., Dep. Biochem. Mol. Biol., Dep. Microbiol. Immunology, Univ. British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Cell (Cambridge, Massachusetts) (1997), 91(4), 511-520
CODEN: CELLB5; ISSN: 0092-8674
PUBLISHER: Cell Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enteropathogenic Escherichia coli (EPEC) belongs to a group of **bacterial pathogens** that induce epithelial cell actin rearrangements resulting in pedestal formation beneath adherent **bacteria**. This requires the secretion of specific virulence proteins needed for signal transduction and intimate adherence. EPEC interaction induces tyrosine phosphorylation of a protein in the **host** membrane, Hp90, which is the **receptor** for the EPEC outer membrane protein, intimin. Hp90-intimin interaction is essential for intimate attachment and pedestal formation. Here, we demonstrate that Hp90 is actually a bacterial protein (Tir). Thus, this **bacterial pathogen** inserts its own **receptor** into mammalian cell surfaces, to which it then adheres to trigger addnl. **host** signaling events and actin nucleation. It is also tyrosine-phosphorylated upon transfer into the host cell.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 21 OF 23 EMBASE  on STN
ACCESSION NUMBER: 97097047 EMBASE
DOCUMENT NUMBER: 1997097047
TITLE: Interactions between enteropathogenic Escherichia coli and host epithelial cells.
AUTHOR: Donnenberg M.S.; Kaper J.B.; **Finlay B.B.**
CORPORATE SOURCE: M.S. Donnenberg, Division of Infectious Diseases, Department of Medicine, Univ. of Maryland Sch. of Medicine, Baltimore, MD 21201, United States. mdonnenb@umabnet.ab.umd.edu
SOURCE: Trends in Microbiology, (1997) 5/3 (109-114). Refs: 49
ISSN: 0966-842X CODEN: TRMIEA
PUBLISHER IDENT.: S 0966-842X(97)01000-7
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The **pathogenesis** of enteropathogenic Escherichia coli (EPEC) infection is emerging as a paradigm for a multistage microorganism-**host** cell interaction. Both type IV fimbriae and a type III secretion apparatus play principal roles in interactions between the **bacteria** and **host** cells. Recent data suggest that **bacteria**-induced signal transduction activates the **receptor** that allows tenacious adherence of the **bacteria** to the **host** cell surface.

L15 ANSWER 22 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 96180689 EMBASE
DOCUMENT NUMBER: 1996180689
TITLE: A **pathogenic bacterium** triggers epithelial signals to form a functional **bacterial** receptor that mediates actin pseudopod formation.
AUTHOR: Rosenshine I.; Ruschkowski S.; **Stein M.**; Reinscheid D.J.; Mills S.D.; **Finlay B.B.**
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia,Vancouver, BC V6T 1Z3, Canada
SOURCE: EMBO Journal, (1996) 15/11 (2613-2624). ISSN: 0261-4189 CODEN: EMJODG
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Enteropathogenic E.coli (EPEC) belongs to a group of **bacterial pathogens** that induce actin accumulation beneath adherent **bacteria**. We found that EPEC adherence to epithelial cells mediates the formation of finger-like pseudopods (up to 10 μm) beneath **bacteria**. These actin-rich structures also contain tyrosine phosphorylated **host** proteins concentrated at the pseudopod tip beneath adherent EPEC. Intimate **bacterial** adherence (and pseudopod formation) occurred only after prior **bacterial** induction of tyrosine phosphorylation of an epithelial membrane protein, Hp90, which then associates directly with an EPEC adhesin, intimin. These interactions lead to cytoskeletal nucleation and pseudopod formation. This is the first example of a **bacterial pathogen** that triggers signals in epithelial cells which activates **receptor** binding activity to a specific **bacterial** ligand and subsequent cytoskeletal rearrangement.

L15 ANSWER 23 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 95105426 EMBASE
DOCUMENT NUMBER: 1995105426
TITLE: Targeting of Salmonella typhimurium to vesicles containing lysosomal membrane glycoproteins bypasses compartments with mannose 6-phosphate receptors.
AUTHOR: Garcia-Del Portillo F.; **Finlay B.B.**
CORPORATE SOURCE: Biotechnology Laboratory, Biochemistry/Molecular Biology Dept., University of British Columbia, 6174 University Boulevard,Vancouver, BC V6T 1Z3, Canada
SOURCE: Journal of Cell Biology, (1995) 129/1 (81-97). ISSN: 0021-9525 CODEN: JCLBA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Salmonella typhimurium is an intracellular **bacterial pathogen** that remains enclosed in vacuoles (SCV) upon entry into the **host** cell. In this study we have examined the

intracellular trafficking route of S. typhimurium within epithelial cells. Indirect immunofluorescence analysis showed that **bacteria** initiated fusion with lysosomal membrane glycoprotein (lgp)-containing compartments .apprx.15 min after **bacterial** internalization. This process was completed .apprx.75 min later and did not require microtubules. Cation-independent (CI)- or cation-dependent (CD)-mannose 6-phosphate **receptors** (M6PRs) were not observed at detectable levels in SCV. Lysosomal enzymes showed a different distribution in SCV: lysosomal-acid phosphatase (LAP) was incorporated into these vacuoles with the same kinetics as lgps, while cathepsin D was present in a low proportion (.apprx.30%) of SCV. Uptake experiments with fluid endocytic tracers such as fluorescein-dextran sulphate (F-DX) or horseradish-peroxidase (HRP) showed that after 2 h of uptake, F-DX was present in .apprx.75% of lgp- containing vesicles in uninfected cells, while only .apprx.15% of SCV contained small amounts of the tracer during the same uptake period. SCV also showed only partial fusion with HRP-preloaded secondary lysosomes, with .apprx.30% of SCV having detectable amounts of HRP at 6 h after infection. These results indicate that SCV show limited accessibility to fluid endocytic tracers and mature lysosomes, and am therefore functionally separated from the endocytic route. Moreover, the unusual intracellular trafficking route of S. typhimurium inside epithelial cells has allowed us to establish the existence of two different lgp-containing vesicles in Salmonella-infected cells: one population is separated from the endocytic route, fusogenic with incoming SCV and may arise from a secretory pathway, while the second involves the classical secondary or mature lysosomes.

FILE 'HOME' ENTERED AT 10:15:33 ON 17 DEC 2003

L13 ANSWER 27 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:256127 CAPLUS
DOCUMENT NUMBER: 131:56220
TITLE: **Binding** of **intimin** from **enteropathogenic** Escherichia **coli** to **Tir** and to host cells
AUTHOR(S): Hartland, Elizabeth L.; Batchelor, Miranda; Delahay, M.; Hale, Christine; Matthews, Stephen; Dougan, Gordon; Knutton, Stuart; Connerton, Ian; Frankel, Gad
CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7 2AZ, UK
SOURCE: Mol. Microbiol. (1999), 32(1), 151-158
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) induce characteristic **attaching** and **effacing** (**A/E**) lesions on epithelial cells. This event is mediated, in part, by **binding** of the bacterial outer membrane **protein**, **intimin**, to a second **EPEC protein**, **Tir** (**translocated intimin receptor**), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study, we have localized the **intimin-binding** domain of **Tir** to a central 107-amino-acid region, designated **Tir**-M. We provide evidence that both the amino- and carboxy-termini of **Tir** are located within the host cell. In addn., using immunogold labeling electron microscopy, we have confirmed that **intimin** can **bind** independently to host cells even in the absence of **Tir**. This **Tir**-independent interaction and the ability of **EPEC** to induce **A/E** lesions requires an intact lectin-like module residing at the carboxy-terminus of the **intimin polypeptide**. Using the yeast two-hybrid system and gel overlays, we show that **intimin** can **bind** both **Tir** and **Tir**-M even when the lectin-like domain is disrupted. These data provide strong evidence that **intimin** interacts not only with **Tir** but also in a lectin-like manner with a host cell **intimin** receptor.

REFERENCE COUNT: 26
REFERENCE(S):
(1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
(2) Deibel, C; Mol Microbiol 1998, V28, P463

CAPLUS
(3) Everest, P; FEMS Microbiol Lett 1995, V126, P97 CAPLUS
(4) Frankel, G; Infect Immun 1994, V62, P1835 CAPLUS
(5) Frankel, G; Infect Immun 1995, V63, P4323 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 28 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:244227 CAPLUS
DOCUMENT NUMBER: 131:41221
TITLE: Structure of the cell-adhesion fragment of **intimin** from **enteropathogenic** Escherichia **coli**
AUTHOR(S): Kelly, Geoff; Prasannan, Sunil; Daniell, Sarah; Fleming, Keiran; Frankel, Gad; Dougan, Gordon; Connerton, Ian; Matthews, Stephen
CORPORATE SOURCE: Department of Biochemistry and Centre for Structural Biology, Imperial College of Science, Technology and Medicine, London, SW7 2AY, UK
SOURCE: Nat. Struct. Biol. (1999), 6(4), 313-318
CODEN: NSBIEW; ISSN: 1072-8368
PUBLISHER: Nature America
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Enteropathogenic** Escherichia **coli** (**EPEC**) induce gross cytoskeletal rearrangement within epithelial cells, immediately beneath the attached bacterium. The C-terminal 280 amino acid residues of **intimin** (Int280; 30.1 kDa), a bacterial cell-adhesion mol., mediate the intimate bacterial host-cell interaction. Recently, interest in this process has been stimulated by the discovery that the bacterial **intimin** receptor **protein** (**Tir**) is translocated into the host cell membrane, phosphorylated, and after **binding** **intimin** triggers the intimate attachment. Using multidimensional NMR and combining perdeuteration with site-specific protonation of Me groups, we have detd. the global fold of Int280. This represents one of the largest, non-oligomeric **protein** structures to be detd. by NMR that has not been previously resolved by X-ray crystallog. Int280 comprises three domains; two Ig-like domains and a C-type lectin-like module, which define a new family of bacterial adhesion mols. These findings also imply that carbohydrate recognition may be important in **intimin** -mediated cell adhesion.
REFERENCE COUNT: 46
REFERENCE(S): (1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS

(3) Casasnovas, J; Proc Natl Acad Sci USA 1998, V95, P4134 CAPLUS
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(5) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS
(6) Delaglio, F; J Biomol NMR 1995, V6, P277 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 29 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:159076 CAPLUS
DOCUMENT NUMBER: 130:308856
TITLE: Phosphorylation of tyrosine 474 of the **enteropathogenic** Escherichia **coli** (**EPEC**) **Tir** receptor molecule is essential for actin nucleating activity and is preceded by additional host modifications
AUTHOR(S): Kenny, Brendan
CORPORATE SOURCE: Department of Pathology and Microbiology, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK
SOURCE: Mol. Microbiol. (1999), 31(4), 1229-1241
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The **enteropathogenic** Escherichia **coli** (**EPEC**) **Tir protein** becomes tyrosine phosphorylated in host cells and displays an increase in apparent mol. mass. The interaction of **Tir** with the **EPEC** outer membrane **protein**, **intimin**, triggers actin nucleation beneath the adherent bacteria. The **enterohaemorrhagic** E. **coli** 0157:H7 (**EHEC**) **Tir** mol. is not tyrosine phosphorylated. In this paper, **Tir** tyrosine phosphorylation is shown to be essential for actin nucleation activity, but not for the increase in apparent mol. mass obsd. in target cells. Tyrosine phosphorylation had no role in **Tir** mol. mass shift, indicating addnl. host modifications. Anal. of **Tir** intermediates indicates that tyrosine-independent modification functions to direct **Tir** 's correct insertion from the cytoplasm into the host membrane. Deletion anal. identified **Tir** domains participating in translocation, assocn. with the host membrane, modification and antibody recognition. **Intimin** was found to **bind** a 55-amino-acid region (TIBA) within **Tir** that topol. and sequence anal. suggests is located in an extracellular loop.

Homologous TIBA sequences exist in integrins, which also **bind intimin**. Collectively, this study provides definitive evidence for the importance of tyrosine phosphorylation for **EPEC Tir** function and reveals differences in the pathogenicity of **EPEC** and **EHEC**. The data also suggest a mechanism for **Tir** insertion into the host membrane, as well as providing clues to the mode of **intimin** -integrin interaction.

REFERENCE COUNT: 33

REFERENCE(S):
(1) Abe, A; Infect Immun 1997, V65, P3547 CAPLUS
(2) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 30 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:83261 CAPLUS

DOCUMENT NUMBER: 130:236380

TITLE: **Enteropathogenic** Escherichia **coli** inhibits phagocytosis

AUTHOR(S): Goosney, Danika L.; Celli, Jean; Kenny, Brendan; Finlay, B. Brett

CORPORATE SOURCE: Biotechnology Laboratory and Departments of Microbiology & Immunology and of Biochemistry & Molecular Biology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Infect. Immun. (1999), 67(2), 490-495
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) interacts with intestinal epithelial cells, activating host signaling pathways leading to cytoskeletal rearrangements and ultimately diarrhea. Here it is shown that **EPEC** interacts with the macrophage-like cell line J774A.1 to inhibit phagocytosis by these cells. Antiphagocytic activity was also obsd. in cultured RAW macrophage-like cells upon **EPEC** infection. The **EPEC** antiphagocytic phenotype was dependent on the type III secretion pathway of **EPEC** and its secreted **proteins**, including EspA, EspB, and EspD. **Intimin** and **Tir** mutants displayed intermediate antiphagocytic activity, suggesting that intimate attachment mediated by

**intimin-Tir binding** may also play a role in antiphagocytosis. Tyrosine dephosphorylation of several host **proteins** was obsd. following infection with secretion-competent **EPEC** but not with secretion-deficient mutants. Dephosphorylation was detectable 120 min after infection with **EPEC**, directly correlating with the onset of the antiphagocytic phenotype. Inhibition of **protein** tyrosine phosphatases by pervanadate treatment increased the no. of intracellular wild-type **EPEC** organisms to levels seen with secretion-deficient mutants, suggesting that dephosphorylation events are linked to the antiphagocytic phenotype. No tyrosine phosphatase activity was detected with the **EPEC**-secreted **proteins**, suggesting that **EPEC** induces antiphagocytosis via a different mechanism than Yersinia species. The present findings demonstrate a novel function for **EPEC** -secreted **proteins** in triggering macrophage **protein** tyrosine dephosphorylation and inhibition of phagocytosis.

REFERENCE COUNT: 31

REFERENCE(S):
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 31 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:711579 CAPLUS

DOCUMENT NUMBER: 130:92716

TITLE: **Translocated intimin receptors** (**Tir**) of Shiga-toxigenic Escherichia coli isolates belonging to serogroups O26, O111, and O157 react with sera from patients with hemolytic-uremic syndrome and exhibit marked sequence heterogeneity

AUTHOR(S): Paton, Adrienne; Manning, Paul A.; Woodrow, Matthew C.; Paton, James C.

CORPORATE SOURCE: Molecular Microbiology Unit, Women's and Children's Hospital, North Adelaide, 5006, Australia

SOURCE: Infect. Immun. (1998), 66(11), 5580-5586
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal
LANGUAGE: English

AB The capacity to form **attaching** and **effacing** ( **A/E**) lesions on the surfaces of enterocytes is an important virulence trait of several enteric pathogens, including **enteropathogenic** Escherichia **coli** (**EPEC**) and Shiga-toxigenic E. coli (STEC). Formation of such lesions depends upon an interaction between a bacterial outer membrane **protein** (**intimin**) and a bacterially encoded receptor **protein** (**Tir**) which is exported from the bacterium and translocated into the host cell membrane. **Intimin**, **Tir**, and several other **proteins** necessary for generation of **A/E** lesions are encoded on a chromosomal pathogenicity island termed the locus for enterocyte effacement (LEE). Reports of sequence heterogeneity and antigenic variation in the region of **intimin** believed to be responsible for receptor **binding** raise the possibility that the receptor itself is also heterogeneous. We have examd. this by cloning and sequencing **tir** genes from three different STEC strains belonging to serogroups O26, O111, and O157. The deduced amino acid sequences for the **Tir** homologs from these strains varied markedly, exhibiting only 65.4, 80.2, and 56.7% identity, resp., to that recently reported for **EPEC** **Tir**. STEC **Tir** is also highly immunogenic in humans. Western blots of E. coli DH5.alpha. expressing the various STEC **tir** genes cloned in pBluescript [but not E. coli DH5.alpha.(pBluescript)] reacted strongly with convalescent sera from patients with hemolytic-uremic syndrome (HUS) caused by known LEE-pos. STEC. Moreover, no reaction was seen when the various clone lysates were probed with serum from a patient with HUS caused by a LEE-neg. STEC or with serum from a healthy individual. Covariation of exposed epitopes on both **intimin** and **Tir** may be a means whereby STEC avoid host immune responses without compromising adhesin-receptor interaction.

IT **207309-52-2**, **Intimin** (Escherichia coli serotype O111:H gene eaeA) **207310-47-2**, **Intimin** receptor (Escherichia coli serotype O111:H translocated) **207998-20-7** **219523-39-4** **219523-42-9**
RL: PRP (Properties)
(amino acid sequence; **translocated intimin** **receptors** (**Tir**) of Shiga-toxigenic Escherichia coli isolates react with sera from patients with hemolytic-uremic syndrome and exhibit marked sequence heterogeneity)

REFERENCE COUNT: 33
REFERENCE(S):
(1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
(2) Altschul, S; J Mol Biol 1990, V215, P403 CAPLUS

(3) Bairoch, A; Nucleic Acids Res 1992, V20, P2013 CAPLUS
(5) Beebakhee, G; FEMS Microbiol Lett 1992, V91, P63 CAPLUS
(7) Butterton, J; Infect Immun 1997, V65, P2127 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 32 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:408570 CAPLUS
DOCUMENT NUMBER: 127:134079
TITLE: **Intimin**-dependent **binding** of **enteropathogenic** Escherichia **coli** to host cells triggers novel signaling events, including tyrosine phosphorylation of phospholipase C-.gamma.1
AUTHOR(S): Kenny, Brendan; Finlay, B. Brett
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Infect. Immun. (1997), 65(7), 2528-2536
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) interactions with HeLa epithelial cells induced the tyrosine phosphorylation of a host **protein** of approx. 150 kDa, Hp150. Phosphorylation of this **protein** band was dependent on the interaction of the **EPEC protein** **intimin** with epithelial cell surfaces and was correlated with pedestal formation. Hp150 phosphorylation was specifically inhibited by the addn. of cytochalasin D, an inhibitor of actin polymn., although this appeared to be an indirect effect preventing interaction of **intimin** with its receptor, tyrosine-phosphorylated **Hp90**, and thus triggering Hp150 phosphorylation. This suggests the involvement of an actin-based movement of membrane-bound tyrosine-phosphorylated **Hp90** to allow its interaction with **intimin**. Anal. of the tyrosine-phosphorylated Hp150 **protein** demonstrated that it is heterogeneous in compn., with phospholipase C-.gamma.1 (PLC-.gamma.1) being a minor component. Activation of PLC-.gamma.1 by tyrosine phosphorylation leads to inositol triphosphate and Ca2+ fluxes, events detected following **EPEC** infection. **EPEC** also induced tyrosine dephosphorylation of host **proteins**, including a 240-kDa host **protein** (Hp240), following **EPEC** infection. **Protein** dephosphorylation appears to be a signaling event which occurs independently of **intimin**. Inhibition of host tyrosine

dephosphorylation events by the addn. of the tyrosine phosphatase inhibitor sodium vanadate did not prevent actin accumulation beneath the adherent bacteria. The authors conclude that **EPEC** induces two sets of signaling events following infection. One set is dependent on **EPEC proteins** secreted by the type III secretion pathway (EspA and EspB) which induces **Hp90** tyrosine phosphorylation and dephosphorylation of host phosphotyrosine **proteins**. The second set, which is also dependent on the first signaling events, requires **intimin** interaction with its receptor, tyrosine-phosphorylated **Hp90** , to trigger Hp150 and PLC-.gamma.1 tyrosine phosphorylation as well as pedestal formation. The second set, which is also dependent on the first signaling events, requires **intimin** interaction with its receptor, tyrosine-phosphorylated **Hp90**, to trigger Hp150 and PLC-.gamma.1 tyrosine phosphorylation as well as pedestal formation.

L13 ANSWER 33 OF 34 CAPLUS 
ACCESSION NUMBER: 1996:381158 CAPLUS
DOCUMENT NUMBER: 125:53467
TITLE: A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation
AUTHOR(S): Rosenshine, Ilan; Ruschkowski, Sharon; Stein, Markus; Reinscheid, Dieter J.; Mills, Scott D.; Finlay, B. Brett
CORPORATE SOURCE: Department Biotechnology and Molecular Genetics, Hebrew University, Jerusalem, 12272, Israel
SOURCE: EMBO J. (1996), 15(11), 2613-2624
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Enteropathogenic** Escherichia **coli** (**EPEC** ) belongs to a group of bacterial pathogens that induce actin accumulation beneath adherent bacteria. We found that **EPEC** adherence to epithelial cells mediates the formation of finger-like pseudopods (up to 10 .mu.m) beneath bacteria. These actin-rich structures also contain tyrosine phosphorylated host **proteins** concd. at the pseudopod tip beneath adherent **EPEC**. Intimate bacterial adherence (and pseudopod formation) occurred only after prior bacterial induction of tyrosine phosphorylation of an epithelial membrane **protein**, **Hp90**, which then assocs. directly with an **EPEC** adhesin, **intimin**. These interactions lead to cytoskeletal nucleation and pseudopod formation. This is the first example of a bacterial pathogen that triggers signals in epithelial cells which activates receptor **binding** activity to a specific bacterial ligand and subsequent cytoskeletal rearrangement.

L13 ANSWER 34 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:129653 CAPLUS
DOCUMENT NUMBER: 124:226331
TITLE: Expression of **attaching/effacing** activity by **enteropathogenic** Escherichia **coli** depends on growth phase, temperature, and **protein** synthesis upon contact with epithelial cells
AUTHOR(S): Rosenshine, Ilan; Ruschkowski, Sharon; Finlay, B. Brett
CORPORATE SOURCE: Fac. Medicine, Hebrew Univ., Jerusalem, 91120, Israel
SOURCE: Infect. Immun. (1996), 64(3), 966-73
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Enteropathogenic** Escherichia **coli** (**EPEC**) induces tyrosine phosphorylation of a **90-kDa protein** (**Hp90**) in infected epithelial cells. This in turn facilitates intimate **binding** of **EPEC** via the outer membrane **protein intimin**, effacement of host cell microvilli, cytoskeletal rearrangement, and bacterial uptake. This phenotype has been commonly referred to as **attaching/effacing** (**A/E**). The ability of **EPEC** to induce **A/E** lesions was dependent on bacterial growth phase and temp. Early-logarithmic-phase **EPEC** grown at 37.degree. elicits strong **A/E** activity within minutes after infection of HeLa epithelial cells. **EPEC** de novo **protein** synthesis during the first minutes of interaction with the host cell was required to elicit **A/E** lesions. However, once formed, bacterial viability was not needed to maintain **A/E** lesions. The type of growth media and partial O2 pressure level do not seem to affect the ability of **EPEC** to cause **A/E** lesions. These results indicates that the **A/E** activity of **EPEC** is tightly regulated by environmental and host factors.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, PHIC, PHIN, TOXLIT, TOXLINE' ENTERED AT 14:23:09 ON 28 SEP 2001)
L14 128 S L13
L15 43 DUP REM L14 (85 DUPLICATES REMOVED)

L15 ANSWER 1 OF 43 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2001454848 MEDLINE

DOCUMENT NUMBER: 21391821 PubMed ID: 11500434
TITLE: **Intimin**-specific immune responses prevent bacterial colonization by the **attaching-effacing** pathogen Citrobacter rodentium.
AUTHOR: Ghaem-Maghami M; Simmons C P; Daniell S; Pizza M; Lewis D; Frankel G; Dougan G
CORPORATE SOURCE: Centre for Molecular Microbiology and Infection, Department of Biochemistry, Imperial College of Science, Technology and Medicine, South Kensington, London SW7 2AZ, United Kingdom.
SOURCE: INFECTION AND IMMUNITY, (2001 Sep) 69 (9) 5597-605. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200109
ENTRY DATE: Entered STN: 20010814
Last Updated on STN: 20010917
Entered Medline: 20010913

AB The formation of **attaching** and **effacing** (**A/E**) lesions on gut enterocytes is central to the pathogenesis of **enterohemorrhagic** (**EHEC**) Escherichia **coli**, **enteropathogenic** E. **coli** (**EPEC**), and the rodent pathogen Citrobacter rodentium. Genes encoding **A/E** lesion formation map to a chromosomal pathogenicity island termed the locus of enterocyte effacement (LEE). Here we show that the LEE-encoded **proteins** EspA, EspB, **Tir**, and **intimin** are the targets of long-lived humoral immune responses in C. rodentium-infected mice. Mice infected with C. rodentium developed robust acquired immunity and were resistant to reinfection with wild-type C. rodentium or a C. rodentium derivative, DBS255(pCVD438), which expressed **intimin** derived from **EPEC** strain E2348/69. The receptor-**binding** domain of **intimin polypeptides** is located within the carboxy-terminal 280 amino acids (Int280). Mucosal and systemic vaccination regimens using enterotoxin-based adjuvants were employed to elicit immune responses to recombinant Int280alpha from **EPEC** strain E2348/69. Mice vaccinated subcutaneously with Int280alpha, in the absence of adjuvant, were significantly more resistant to oral challenge with DBS255(pCVD438) but not with wild-type C. rodentium. This type-specific immunity could not be overcome by employing an exposed, highly conserved domain of **intimin** (Int388-667) as a vaccine. These results show that anti-**intimin** immune responses can modulate the outcome of a C. rodentium infection and support the use of **intimin** as a component of a type-specific **EPEC** or **EHEC**

vaccine.

L15 ANSWER 2 OF 43 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2001248137 MEDLINE
DOCUMENT NUMBER: 21189250 PubMed ID: 11292754
TITLE: Recruitment of cytoskeletal and signaling **proteins** to **enteropathogenic** and **enterohemorrhagic** Escherichia **coli** pedestals.
AUTHOR: Goosney D L; DeVinney R; Finlay B B
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3.
SOURCE: INFECTION AND IMMUNITY, (2001 May) 69 (5) 3315-22. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010517
Last Updated on STN: 20010517
Entered Medline: 20010510

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) is a human pathogen that attaches to intestinal epithelial cells and causes chronic watery diarrhea. A close relative, **enterohemorrhagic** E. **coli** (**EHEC**), causes severe bloody diarrhea and hemolytic-uremic syndrome. Both pathogens insert a **protein**, **Tir**, into the host cell plasma membrane where it **binds intimin**, the outer membrane ligand of **EPEC** and **EHEC**. This interaction triggers a cascade of signaling events within the host cell and ultimately leads to the formation of an actin-rich pedestal upon which the pathogen resides. Pedestal formation is critical in mediating **EPEC**- and **EHEC**-induced diarrhea, yet very little is known about its composition and organization. In **EPEC**, pedestal formation requires **Tir** tyrosine 474 phosphorylation. In **EHEC Tir** is not tyrosine phosphorylated, yet the pedestals appear similar. The composition of the **EPEC** and **EHEC** pedestals was analyzed by examining numerous cytoskeletal, signaling, and adapter **proteins**. Of the 25 **proteins** examined, only two, calpactin and CD44, were recruited to the site of bacterial attachment independently of **Tir**. Several others, including ezrin, talin, gelsolin, and tropomyosin, were recruited to the site of **EPEC** attachment independently of **Tir** tyrosine 474 phosphorylation but required **Tir** in the host membrane. The remaining **proteins** were recruited to the pedestal in a

manner dependent on **Tir** tyrosine phosphorylation or were not recruited at all. Differences were also found between the **EPEC** and **EHEC** pedestals: the adapter **proteins** Grb2 and CrkII were recruited to the **EPEC** pedestal but were absent in the **EHEC** pedestal. These results demonstrate that although **EPEC** and **EHEC** recruit similar cytoskeletal **proteins**, there are also significant differences in pedestal composition.

L15 ANSWER 3 OF 43 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2001490958 IN-PROCESS
DOCUMENT NUMBER: 21424752 PubMed ID: 11533668
TITLE: **Enteropathogenic** E. **coli** **Tir binds** Nck to initiate actin pedestal formation in host cells.
AUTHOR: Gruenheid S; DeVinney R; Bladt F; Goosney D; Gelkop S; Gish G D; Pawson T; Finlay B B
CORPORATE SOURCE: [1] Biotechnology Laboratory, University of British Columbia, 6174 University Boulevard, Vancouver V6T 1G3, Canada [2] These authors contributed equally to this work.
SOURCE: NATURE CELL BIOLOGY, (2001 Sep) 3 (9) 856-9. Journal code: DIQ; 100890575. ISSN: 1465-7392.
PUB. COUNTRY: England: United Kingdom Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20010905 Last Updated on STN: 20010905

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) is a bacterial pathogen that causes infantile diarrhea worldwide. **EPEC** injects a bacterial **protein**, **translocated intimin receptor** (**Tir**), into the host-cell plasma membrane where it acts as a receptor for the bacterial outer membrane **protein**, **intimin**. The interaction of **Tir** and **intimin** triggers a marked rearrangement of the host actin cytoskeleton into pedestals beneath adherent bacteria. On delivery into host cells, **EPEC Tir** is phosphorylated on tyrosine 474 of the intracellular carboxy-terminal domain, an event that is required for pedestal formation. Despite its essential role, the function of **Tir** tyrosine phosphorylation has not yet been elucidated. Here we show that tyrosine 474 of **Tir** directly **binds** the host-cell adaptor **protein** Nck, and that Nck is required for the recruitment of both neural Wiskott-Aldrich-syndrome **protein** (N-WASP) and the actin-related **protein** (Arp)2/3 complex to the **EPEC** pedestal, directly linking **Tir** to the cytoskeleton. Cells

with null alleles of both mammalian Nck genes are resistant to the effects of **EPEC** on the actin cytoskeleton. These results implicate Nck adaptors as host-cell determinants of **EPEC** virulence.

L15 ANSWER 4 OF 43 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2001217117 MEDLINE
DOCUMENT NUMBER: 21204341 PubMed ID: 11310447
TITLE: **Intimin** from Shiga toxin-producing Escherichia coli and its isolated C-terminal domain exhibit different **binding** properties for **Tir** and a eukaryotic surface receptor.
AUTHOR: Deibel C; Dersch P; Ebel F
CORPORATE SOURCE: Institut fur Medizinische Mikrobiologie, Justus-Liebig-Universitat, Giessen, Germany.
SOURCE: INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, (2001 Mar) 290 (8) 683-91.
Journal code: DQD; 100898849. ISSN: 1438-4221.
PUB. COUNTRY: Germany: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 20010820
Last Updated on STN: 20010820
Entered Medline: 20010816

AB The outer membrane **protein intimin** plays a crucial role in the **attaching** and **effacing** process employed by different enteropathogens to colonize the epithelial surface of their hosts. In this study we have characterized the C-terminal **binding** domain of **intimin** from the Shiga toxin-producing Escherichia coli strain 413/89-1, that belongs to the beta-subtype of **intimins**. We found that a fusion of this domain to the maltose-**binding protein binds** efficiently to both the **translocated intimin receptor** (**Tir**) and the surface of uninfected eukaryotic host cells. In contrast, no such **binding** was observed with the full-length **protein** localized on the bacterial surface. As the C-terminal domain of **intimin** and the full-length **protein** differ in their **binding** activity, we suggest that the **intimin-binding** domain might be controlled by the N-terminal portion of the molecule to prevent unproductive interactions with molecules in the lumen of the gut.

L15 ANSWER 5 OF 43 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 2001:663652 SCISEARCH

THE GENUINE ARTICLE: 462BY
TITLE: The **enterohaemorrhagic** Escherichia **coli** (serotype O157 : H7) **Tir** molecule is not functionally interchangeable for its **enteropathogenic** E-**coli** (serotype O127 : H6) homologue
AUTHOR: Kenny B (Reprint)
CORPORATE SOURCE: Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Univ Walk, Bristol BS8 1TD, Avon, England (Reprint); Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England
COUNTRY OF AUTHOR: England
SOURCE: CELLULAR MICROBIOLOGY, (AUG 2001) Vol. 3, No. 8, pp. 499-510.
Publisher: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND.
ISSN: 1462-5814.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 41
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB A major virulence determinant of enteropathogenic Escherichia coil (**EPEC**) is the **Tir** molecule that is translocated into the plasma membrane where it orchestrates cytoskeletal rearrangements. **Tir** undergoes several phosphorylation events within host cells, with modification on a tyrosine essential for its actin-nucleating function. The **EHEC** (serotype O157:H7) **Tir** homologue is not tyrosine phosphorylated implying that it uses an alternative mechanism to nucleate actin. This is supported in this study by the demonstration that **EHEC Tir** is unable to functionally substitute for its **EPEC** homologue. Like **EPEC**, the **EHEC Tir** molecule is phosphorylated within host cells, with the actin-nucleating dysfunction correlated to an altered modification profile. In contrast to **EHEC Tir**, the **EPEC Tir** molecule mediated actin nucleation whether delivered into host cells by either strain. Thus, it would appear that **EHEC** encodes specific factor(s) that facilitate the correct modification of its **Tir** molecule within host cells. Domain-swapping experiments revealed that the N-terminal, alpha -actinin **binding**, **Tir** domains were functionally interchangeable, with both the actin-nucleating dysfunction and altered modification profiles linked to the **EHEC** C-terminal **Tir** domain. This tyrosine-independent modification process presumably confers an advantage to **EHEC** O157:H7 and may contribute to the prevalence of this strain in **EHEC** disease. The presented data are also consistent with

**EPEC** and **EHEC** sharing non-phosphotyrosine phosphorylation event(s), with an important role for such modifications in **Tir** function. An **EHEC**-induced phosphotyrosine dephosphorylation activity is also identified.

L15 ANSWER 6 OF 43 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 2001371277 MEDLINE
DOCUMENT NUMBER: 21235726 PubMed ID: 11336837
TITLE: **Intimin** and the host cell--is it bound to end in **Tir**(s)?.
AUTHOR: Frankel G; Phillips A D; Trabulsi L R; Knutton S; Dougan G; Matthews S
CORPORATE SOURCE: Centre for Molecular Microbiology and Infection, Dept. of Biochemistry, Imperial College of Science, Technology and Medicine, SW7 2AZ, London, UK.. g.frankel@ic.ac.uk
SOURCE: TRENDS IN MICROBIOLOGY, (2001 May) 9 (5) 214-8. Ref: 47
Journal code: B1N; 9310916. ISSN: 0966-842X.
PUB. COUNTRY: England: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010702
Last Updated on STN: 20010702
Entered Medline: 20010628

AB Intimate bacterial adhesion to the intestinal epithelium is a pathogenic mechanism shared by several human and animal enteric pathogens, including **enteropathogenic** and **enterohaemorrhagic** Escherichia **coli**. Two bacterial **protein** partners involved in this intimate association have been identified, **intimin** and **Tir**. Some key remaining questions include whether **intimin** specifically interacts with one or more host-cell-encoded molecules and whether these contacts are a prerequisite for the subsequent intimate **intimin-Tir** association. Recent data support the hypothesis that the formation of a stable **intimin-Tir** relationship is the consequence of **intimin** **protein** interactions involving both host and bacterial components.

L15 ANSWER 7 OF 43 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 2001287659 MEDLINE
DOCUMENT NUMBER: 21195867 PubMed ID: 11298278
TITLE: Site-directed mutagenesis of **intimin** alpha

modulates **intimin**-mediated tissue tropism and host specificity.

AUTHOR: Reece S; Simmons C P; Fitzhenry R J; Matthews S; Phillips A D; Dougan G; Frankel G

CORPORATE SOURCE: Centre for Molecular Microbiology and Infection, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London SW7 2AZ, UK.

SOURCE: MOLECULAR MICROBIOLOGY, (2001 Apr) 40 (1) 86-98. Journal code: MOM; 8712028. ISSN: 0950-382X.

PUB. COUNTRY: England: United Kingdom
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200106

ENTRY DATE: Entered STN: 20010618
Last Updated on STN: 20010618
Entered Medline: 20010614

AB The hallmark of **enteropathogenic** (**EPEC**) and **enterohaemorrhagic** (**EHEC**) Escherchia **coli** adhesion to host cells is intimate attachment leading to the formation of distinctive '**attaching** and **effacing** ' lesions. This event is mediated, in part, by **binding** of the bacterial adhesion molecule **intimin** to a second bacterial **protein**, **Tir**, delivered by a type III secretion system into the host cell plasma membrane. The receptor-**binding** activity of **intimin** is localized to the C-terminal 280 amino acids (Int280) and at least five distinct **intimin** types (alpha, beta, gamma, delta and epsilon) have been identified thus far. In addition to **binding** to **Tir**, **intimin** can also **bind** to a component encoded by the host. The consequence of latter **intimin-binding** activity may determine tissue tropism and host specificity. In this study we selected three amino acids in **intimin**, which are implicated in **Tir binding**, for site-directed mutagenesis. We used the yeast two-hybrid system and gel overlays to study **intimin-Tir protein** interaction. In addition, the biological consequences of the mutagenesis was tested using a number of infection models (cultured epithelial cells, human intestinal explants and a mouse model). We report that while an I237/897A substitution (positions numbered according to Int280alpha/whole **intimin** alpha) in **intimin** alpha did not have any affect on its biological activity, a T255/914A substitution attenuated **intimin** activity in vivo. In contrast, the mutation V252/911A affected tissue targeting in the human intestinal explant model and attenuated the biological activity of **intimin** in the mouse model. This study provides the first clues of the molecular basis of how **intimin** mediates

tissue tropism and host specificity.

L15 ANSWER 8 OF 43 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-499357 [44] WPIDS
DOC. NO. NON-CPI: N2000-370118
DOC. NO. CPI: C2000-149915
TITLE: Screening for inhibitors of **intimin binding** to eukaryotic cells, for use in diagnosing, preventing and treating bacterial infections, especially Escherichia coli O157 H7.
DERWENT CLASS: B04 D13 D16 S03
INVENTOR(S): DOUGAN, G; FRANKEL, G M; HALE, C B; MATTHEWS, S J
PATENT ASSIGNEE(S): (IMCO-N) IMPERIAL COLLEGE INNOVATIONS LTD
COUNTRY COUNT: 90
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 2000045173 | A1 | 20000803 | (200044)* | EN | 94 |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW | | | | | |
| W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW | | | | | |
| AU 2000021205 | A | 20000818 | (200057) | | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 2000045173 | A1 | WO 2000-GB254 | 20000131 |
| AU 2000021205 | A | AU 2000-21205 | 20000131 |

FILING DETAILS:

| PATENT NO | KIND | PATENT NO |
|---|---|---|
| AU 2000021205 | A Based on | WO 200045173 |

PRIORITY APPLN. INFO: GB 1999-1897 19990129
AN 2000-499357 [44] WPIDS
AB WO 200045173 A UPAB: 20000913
NOVELTY - Screening for an inhibitor of **intimin binding** to eukaryotic cells, comprising exposing an **intimin polypeptide** having a **Tir** -independent cell **binding** activity to test agents, and obtaining an inhibitor based on its ability to **bind** the

**polypeptide**, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) screening for an inhibitor of **intimin binding** to eukaryotic cells, comprising exposing a **polypeptide** comprising the **intimin Tir binding** domain to test agents, and obtaining an inhibitor which is not **Tir**, based on its ability to **bind** the domain;

(2) an inhibitor obtained by the novel method, or the method of (1);

(3) an inhibitor of **intimin binding** to eukaryotic cells, which comprises an O-linked sugar residue which is exposed on a mammalian cell, and which is used to produce a medical composition;

(4) a food product, comprising a foodstuff and an inhibitor of (2) or (3);

(5) a composition comprising a carrier or diluent, and an inhibitor of (2) or (3);

(6) sorting cells, comprising identifying and/or separating cells based on their ability to **bind** an **intimin polypeptide** having a **Tir**-dependent, or independent, cell **binding** activity; and

(7) screening for an inhibitor of **intimin binding** to a eukaryotic cell, preferably an intestinal epithelial cell.

ACTIVITY - Antibacterial. No biological data is given.

MECHANISM OF ACTION - **Intimin binding** to eukaryotic cell inhibitor.

USE - The inhibitors are used in the prevention, treatment and/or diagnosis of bacterial infections, preferably by enteropathic and/or **enterohemorrhagic** Escherichia **coli**, Shiga toxigenic **E. coli**, Hafnia alvei or Citrobacter freundii, or especially **E.** coli O157:H7. The infections cause a histopathological effect known as attachment and effacement on intestinal epithelial cells. The inhibitors can be used to produce food supplements or additives, especially where the food is a milk substitute. The methods can be used to sort cells based on their ability to **bind** to a **Tir** independent cell **binding** domain of an **intimin polypeptide**. (All claimed). **Polypeptides** having **Tir**-independent **intimin binding** activity can be used to produce a vaccine against a bacterial disease.

Dwg.0/9

L15 ANSWER 9 OF 43 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 2000:616771 SCISEARCH
THE GENUINE ARTICLE: 341UL

TITLE: Exploitation of host cells by **enteropathogenic** Escherichia **coli**
AUTHOR: Vallance B A; Finlay B B (Reprint)
CORPORATE SOURCE: UNIV BRITISH COLUMBIA, BIOTECHNOL LAB, ROOM 237, WESBROOK BLDG, 6174 UNIV BLVD, VANCOUVER, BC V6T 1Z3, CANADA (Reprint); UNIV BRITISH COLUMBIA, BIOTECHNOL LAB, VANCOUVER, BC V6T 1Z3, CANADA
COUNTRY OF AUTHOR: CANADA
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1 AUG 2000) Vol. 97, No. 16, pp. 8799-8806.
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418.
ISSN: 0027-8424.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 60

*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Microbial pathogens have evolved many ingenious ways to infect their hosts and cause disease, including the subversion and exploitation of target host cells. One such subversive microbe is enteropathogenic Escherichia coil (**EPEC**), A major cause of infantile diarrhea in developing countries, **EPEC** poses a significant health threat to children worldwide, Central to **EPEC**-mediated disease is its colonization of the intestinal epithelium. After initial adherence, **EPEC** causes the localized effacement of microvilli and intimately attaches to the host cell surface, forming characteristic **attaching** and **effacing** (**A/E**) lesions. Considered the prototype for a family of **A/E** lesion-causing bacteria, recent in vitro studies of **EPEC** have revolutionized our understanding of how these pathogens infect their hosts and cause disease, Intimate attachment requires the type Ill-mediated secretion of bacterial **proteins**, several of which are translocated directly into the infected cell, including the bacteria's own receptor (**Tir**). **Binding** to this membrane-bound, pathogen-derived **protein** permits **EPEC** to intimately attach to mammalian cells, The translocated **EPEC proteins** also activate signaling pathways within the underlying cell, causing the reorganization of the host actin cytoskeleton and the formation of pedestal-like structures beneath the adherent bacteria, This review explores what is known about **EPEC**'s subversion of mammalian cell functions and how this knowledge has provided novel insights into bacterial pathogenesis and microbe-host interactions, Future studies of **A/E** pathogens in animal models should provide further insights into how **EPEC** exploits not

only epithelial cells but other host cells, including those of the immune system, to cause diarrheal disease.

L15 ANSWER 10 OF 43 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000380418 EMBASE
TITLE: The locus of enterocyte effacement (LEE)-encoded regulator controls expression of both LEE- and non-LEE-encoded virulence factors in **enteropathogenic** and **enterohemorrhagic** Escherichia **coli**.
AUTHOR: Elliott S.J.; Sperandio V.; Giron J.A.; Shin S.; Mellies J.L.; Wainwright L.; Hutcheson S.W.; McDaniel T.K.; Kaper J.B.
CORPORATE SOURCE: J.B. Kaper, Center for Vaccine Development, Univ. of Maryland School of Medicine, 685 W. Baltimore St., Baltimore, MD 21201, United States.
jkaper@umaryland.edu
SOURCE: Infection and Immunity, (2000) 68/11 (6115-6126).
Refs: 48
ISSN: 0019-9567 CODEN: INFIBR
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Regulation of virulence gene expression in **enteropathogenic** Escherichia **coli** (**EPEC**) and **enterohemorrhagic** E. **coli** (**EHEC**) is incompletely understood. In **EPEC**, the plasmid-encoded regulator Per is required for maximal expression of **proteins** encoded on the locus of enterocyte effacement (LEE), and a LEE-encoded regulator (Ler) is part of the Per-mediated regulatory cascade upregulating the LEE2, LEE3, and LEE4 promoters. We now report that Ler is essential for the expression of multiple LEE-located genes in both **EPEC** and **EHEC**, including those encoding the type III secretion pathway, the secreted Esp **proteins**, **Tir**, and **intimin**. Ler is therefore central to the process of **attaching** and **effacing** (AE) lesion formation. Ler also regulates the expression of LEE-located genes not required for AE-lesion formation, including rorf2, orf10, rorf10, orf19, and espF, indicating that Ler regulates additional virulence properties. In addition, Ler regulates the expression of **proteins** encoded outside the LEE that are not essential for AE lesion formation, including TagA in **EHEC** and EspC in **EPEC**. .DELTA.ler mutants of both **EPEC** and **EHEC** show altered adherence to epithelial cells and express novel fimbriae. Ler is therefore a global regulator of virulence gene expression in

**EPEC** and **EHEC**.

L15 ANSWER 11 OF 43 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 2000428062 MEDLINE
DOCUMENT NUMBER: 20407319 PubMed ID: 10948130
TITLE: Human response to Escherichia coli O157:H7 infection: antibodies to secreted virulence factors.
AUTHOR: Li Y; Frey E; Mackenzie A M; Finlay B B
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3.
SOURCE: INFECTION AND IMMUNITY, (2000 Sep) 68 (9) 5090-5. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20000922
Last Updated on STN: 20000922
Entered Medline: 20000908

AB Vaccination has been proposed for the prevention of disease due to **enterohemorrhagic** Escherichia **coli** (**EHEC**), but the immune response following human infection, including the choice of potential antigens, has not been well characterized. To study this, sera were obtained from five pediatric patients with acute diarrhea caused by E. coli O157:H7 0, 8, and 60 days after hospitalization. These sera were used to examine the immune response to four different **EHEC** virulence factors: **Tir** (**translocated intimin receptor**, which is inserted into the host cell membrane), **intimin** (bacterial outer membrane **protein** which **binds** to **Tir**), EspA (secreted **protein** which forms filamentous structures on **EHEC** surface), and EspB (inserted into the host membrane and cytoplasm). The response to O157:H7 lipopolysaccharide was also examined. Sera were assayed against purified recombinant **proteins** using immunoblot analysis and by enzyme-linked immunosorbent assay to determine the sera's titers to each of the antigens in all patients. We found that there was little reaction to EspA, EspB, and **intimin** in the acute-phase sera, although there was some reactivity to **Tir**. By day 8, titers of antibody to all four virulence factors were present in all patients, with a very strong response against **Tir** (up to a titer of 1:256,000), especially in hemolytic-uremic syndrome patients, and lesser strong responses to the other three antigens. The titer to the antigens 60 days after hospitalization was decreased but was still highest for **Tir**. These results suggest that there is a strong immune response to

**Tir**, and to a lesser extent to the other three virulence factors, following **EHEC** disease, indicating that these bacterial molecules are potential vaccine candidates for preventing **EHEC** disease. They also suggest that bacterial virulence factors that are inserted into host cells during infection by type III secretion systems (**Tir** or EspB) are still recognized by the host immune response.

L15 ANSWER 12 OF 43 MEDLINE
ACCESSION NUMBER: 2000404335 MEDLINE
DOCUMENT NUMBER: 20359360 PubMed ID: 10899867
TITLE: Expression of **intimin** gamma from **enterohemorrhagic** Escherichia **coli** in Citrobacter rodentium.
AUTHOR: Hartland E L; Huter V; Higgins L M; Goncalves N S; Dougan G; Phillips A D; MacDonald T T; Frankel G
CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science, Technology and Medicine, London SW7 2AZ, United Kingdom.
SOURCE: INFECTION AND IMMUNITY, (2000 Aug) 68 (8) 4637-46. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000901
Last Updated on STN: 20000901
Entered Medline: 20000824

AB The carboxy-terminal 280 amino acids (Int280) of the bacterial adhesion molecule **intimin** include the receptor-**binding** domain. At least five different types of Int280, designated alpha, beta, gamma, delta, and epsilon, have been described based on sequence variation in this region. Importantly, the **intimin** types are associated with different evolutionary branches and contribute to distinct tissue tropism of **intimin**-positive bacterial pathogens. In this study we engineered a strain of Citrobacter rodentium, which normally displays **intimin** beta, to express **intimin** gamma from **enterohemorrhagic** Escherichia **coli**. We show that **intimin** gamma **binds** to the **translocated intimin receptor** ( **Tir**) from C. rodentium and has the ability to produce **attaching** and **effacing** lesions on HEp-2 cells. However, C. rodentium expressing **intimin** gamma could not colonize orally infected mice or induce mouse colonic hyperplasia. These results suggest that **intimin** may contribute to host specificity, possibly through its interaction with a receptor on the

host cell surface.

L15 ANSWER 13 OF 43 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 2000316068 MEDLINE
DOCUMENT NUMBER: 20316068 PubMed ID: 10858257
TITLE: Mechanical fractionation reveals structural requirements for **enteropathogenic** Escherichia **coli Tir** insertion into host membranes.
AUTHOR: Gauthier A; de Grado M; Finlay B B
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology and Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada.
SOURCE: INFECTION AND IMMUNITY, (2000 Jul) 68 (7) 4344-8. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000728
Last Updated on STN: 20000728
Entered Medline: 20000720

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) inserts its receptor for intimate adherence (**Tir**) into host cell membranes by using a type III secretion system. Detergents are frequently used to fractionate infected host cells to investigate bacterial **protein** delivery into mammalian cells. In this study, we found that the Triton X-100-soluble membrane fraction from **EPEC**-infected HeLa cells was contaminated with bacterial **proteins**. We therefore applied a mechanical method of cell lysis and ultracentrifugation to fractionate infected HeLa cells to investigate the biology and biochemistry of **Tir** delivery and translocation. This method demonstrates that the translocation of **Tir** into the host cell membrane requires its transmembrane domains, but not tyrosine phosphorylation or **binding** to **Tir**'s ligand, **intimin**.

L15 ANSWER 14 OF 43 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 2000296671 MEDLINE
DOCUMENT NUMBER: 20296671 PubMed ID: 10835344
TITLE: Structural basis for recognition of the **translocated intimin receptor** (**Tir**) by **intimin** from **enteropathogenic** Escherichia **coli**.

AUTHOR: Batchelor M; Prasannan S; Daniell S; Reece S; Connerton I; Bloomberg G; Dougan G; Frankel G; Matthews S

CORPORATE SOURCE: Department of Biochemistry and Centre for Structural Biology, Imperial College of Science, Technology and Medicine, London SW7 2AZ, UK.

SOURCE: EMBO JOURNAL, (2000 Jun 1) 19 (11) 2452-64. Journal code: EMB; 8208664. ISSN: 0261-4189.

PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: PDB-UNKNOWN

ENTRY MONTH: 200007

ENTRY DATE: Entered STN: 20000728
Last Updated on STN: 20000728
Entered Medline: 20000720

AB **Intimin** is a bacterial adhesion molecule involved in intimate attachment of **enteropathogenic** and **enterohaemorrhagic** Escherichia **coli** to mammalian host cells. **Intimin** targets the **translocated intimin receptor** (**Tir**), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study we localized the **Tir-binding** region of **intimin** to the C-terminal 190 amino acids (Int190). We have also determined the region's high-resolution solution structure, which comprises an immunoglobulin domain that is intimately coupled to a novel C-type lectin domain. This fragment, which is necessary and sufficient for **Tir** interaction, defines a new super domain in **intimin** that exhibits striking structural similarity to the integrin-**binding** domain of the Yersinia invasin and C-type lectin families. The extracellular portion of **intimin** comprises an articulated rod of immunoglobulin domains extending from the bacterium surface, conveying a highly accessible 'adhesive tip' to the target cell. The interpretation of NMR-titration and mutagenesis data has enabled us to identify, for the first time, the **binding** site for **Tir**, which is located at the extremity of the Int190 moiety.

L15 ANSWER 15 OF 43 MEDLINE DUPLICATE 10

ACCESSION NUMBER: 2000384587 MEDLINE

DOCUMENT NUMBER: 20307491 PubMed ID: 10846212

TITLE: **Intimin** from **enteropathogenic** Escherichia **coli** mediates remodelling of the eukaryotic cell surface.

AUTHOR: Phillips A D; Giron J; Hicks S; Dougan G; Frankel G

CORPORATE SOURCE: University Department of Paediatric Gastroenterology,

Royal Free Hospital, London NW3 2QG, UK.
SOURCE: MICROBIOLOGY, (2000 Jun) 146 ( Pt 6) 1333-44.
Journal code: BXW; 9430468. ISSN: 1350-0872.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000818
Last Updated on STN: 20000818
Entered Medline: 20000807

AB Adhesion to cultured epithelial cells by **enteropathogenic** Escherichia **coli** (**EPEC**) is associated with extensive rearrangement of the host cell cytoskeleton. Evidence has been presented that **EPEC** adhesion is associated with activation of signal transduction pathways leading to production of a characteristic histopathological feature known as the **attaching** and **effacing** (**A/E**) lesion. **A/E** lesion formation requires **intimin**, an **EPEC** adhesion molecule and several **EPEC** secreted **proteins** (EspA, B, D and **Tir**) involved in cell signalling and **protein** translocation. In this study it is shown that HEp-2 cells respond during the early stages of infection with two wild-type **EPEC** strains (B171 and E2348/69) by producing microvillus-like processes (MLP) at the site of initial bacterial adherence. **Intimin** appears to play a key role in MLP elongation. At later stages of infection with these wild-type **EPEC** strains, when **A/E** lesions have formed, the MLP were reduced in number and length to appear as at time zero, and the cell surface in the vicinity of bacterial clusters appeared unaffected. In contrast, infection with EspA- or EspB-negative, but **intimin**-positive, **EPEC** strains (UMD872 and UMD864, respectively) resulted in enhanced MLP proliferation and formation of 'cage-like' structures engulfing the bacteria. Inoculating HEp-2 cells with **intimin**-coated latex spheres induced similar 'cage-like' structures. Caco-2 cells did not show **intimin**-induced microvillus elongation in response to **EPEC** infection, although microvillus effacement and reduction in number occurred. Similar phenomena appeared on B171 and E2348/69 infection of paediatric intestine using in vitro organ culture, i.e. elongated microvilli were seen in association with small colonies and at the periphery of large localized colonies, along with evidence of microvillus breakdown and debris in the colony centre. These results show that **intimin** activates signal transduction pathways involved in the remodelling of the eukaryotic cell surface, probably via **binding** to a receptor encoded by the host cell.

L15 ANSWER 16 OF 43 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 2000346505 MEDLINE
DOCUMENT NUMBER: 20346505 PubMed ID: 10890451
TITLE: Crystal structure of **enteropathogenic** Escherichia **coli intimin**-receptor complex.
AUTHOR: Luo Y; Frey E A; Pfuetzner R A; Creagh A L; Knoechel D G; Haynes C A; Finlay B B; Strynadka N C
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada.
SOURCE: NATURE, (2000 Jun 29) 405 (6790) 1073-7.
Journal code: NSC; 0410462. ISSN: 0028-0836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1F00; PDB-1F02
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000811
Last Updated on STN: 20000811
Entered Medline: 20000731

AB **Intimin** and its **translocated intimin receptor** (**Tir**) are bacterial **proteins** that mediate adhesion between mammalian cells and **attaching** and **effacing** (**A/E**) pathogens. **Enteropathogenic** Escherichia **coli** (**EPEC**) causes significant paediatric morbidity and mortality world-wide. A related **A/E** pathogen, **enterohaemorrhagic** E. **coli** (**EHEC**; O157:H7) is one of the most important food-borne pathogens in North America, Europe and Japan. A unique and essential feature of **A/E** bacterial pathogens is the formation of actin-rich pedestals beneath the intimately adherent bacteria and localized destruction of the intestinal brush border. The bacterial outer membrane adhesin, **intimin**, is necessary for the production of the **A/E** lesion and diarrhoea. The **A/E** bacteria translocate their own receptor for **intimin**, **Tir**, into the membrane of mammalian cells using the type III secretion system. The translocated **Tir** triggers additional host signalling events and actin nucleation, which are essential for lesion formation. Here we describe the the crystal structures of an **EPEC intimin** carboxy-terminal fragment alone and in complex with the **EPEC Tir intimin-binding** domain, giving insight into the molecular mechanisms of adhesion of **A/E** pathogens.

L15 ANSWER 17 OF 43 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 2000392530 MEDLINE

DOCUMENT NUMBER: 20334993 PubMed ID: 10873808
TITLE: **Enteropathogenic E. coli translocated intimin receptor, Tir**, interacts directly with alpha-actinin.
AUTHOR: Goosney D L; DeVinney R; Pfuetzner R A; Frey E A; Strynadka N C; Finlay B B
CORPORATE SOURCE: Biotechnology Laboratory, The Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada.
SOURCE: CURRENT BIOLOGY, (2000 Jun 15) 10 (12) 735-8.
Journal code: B44; 9107782. ISSN: 0960-9822.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000824
Last Updated on STN: 20000824
Entered Medline: 20000816

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) triggers a dramatic rearrangement of the host epithelial cell actin cytoskeleton to form an **attaching** and **effacing** lesion, or pedestal. The pathogen remains attached extracellularly to the host cell through the pedestal for the duration of the infection. At the tip of the pedestal is a bacterial **protein, Tir**, which is secreted from the bacterium into the host cell plasma membrane, where it functions as the receptor for an **EPEC** outer membrane **protein, intimin** [1]. Delivery of **Tir** to the host cell results in its tyrosine phosphorylation, followed by **Tir-intimin binding**. **Tir** is believed to anchor **EPEC** firmly to the host cell, although its direct linkage to the cytoskeleton is unknown. Here, we show that **Tir** directly **binds** the cytoskeletal **protein** alpha-actinin. alpha-Actinin is recruited to the pedestal in a **Tir**-dependent manner and colocalizes with **Tir** in infected host cells. **Binding** is mediated through the amino terminus of **Tir**. Recruitment of alpha-actinin occurs independently of **Tir** tyrosine phosphorylation. Recruitment of actin, VASP, and N-WASP, however, is abolished in the absence of this tyrosine phosphorylation. These results suggest that **Tir** plays at least three roles in the host cell during infection: **binding intimin** on **EPEC**; mediating a stable anchor with alpha-actinin through its amino terminus in a phosphotyrosine-independent manner; and recruiting additional cytoskeletal **proteins** at the carboxyl terminus in a phosphotyrosine-dependent manner. These

findings demonstrate the first known direct linkage between extracellular **EPEC**, through the transmembrane **protein Tir**, to the host cell actin cytoskeleton via alpha-actinin.

L15 ANSWER 18 OF 43 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 2001078525 MEDLINE
DOCUMENT NUMBER: 20545157 PubMed ID: 11093251
TITLE: Interaction of the **enteropathogenic** Escherichia **coli protein**, **translocated intimin receptor** (**Tir**), with focal adhesion **proteins**.
AUTHOR: Freeman N L; Zurawski D V; Chowrashi P; Ayoob J C; Huang L; Mittal B; Sanger J M; Sanger J W
CORPORATE SOURCE: Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia 19104-6058, USA.
SOURCE: CELL MOTILITY AND THE CYTOSKELETON, (2000 Dec) 47 (4) 307-18.
Journal code: CRD. ISSN: 0886-1544.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010111

AB When **enteropathogenic** Escherichia **coli** (**EPEC**) attach and infect host cells, they induce a cytoskeletal rearrangement and the formation of cytoplasmic columns of actin filaments called pedestals. The attached **EPEC** and pedestals move over the surface of the host cell in an actin-dependent reaction [Sanger et al., 1996: Cell Motil Cytoskeleton 34:279-287]. The discovery that **EPEC** inserts the **protein**, **translocated intimin receptor** (**Tir**), into the membrane of host cells, where it **binds** the **EPEC** outer membrane **protein**, **intimin** [Kenny et al., 1997: Cell 91:511-520], suggests **Tir** serves two functions: tethering the bacteria to the host cell and providing a direct connection to the host's cytoskeleton. The sequence of **Tir** predicts a **protein** of 56.8 kD with three domains separated by two predicted trans-membrane spanning regions. A GST-fusion **protein** of the N-terminal 233 amino acids of **Tir** (Tir1) **binds** to alpha-actinin, talin, and vinculin from cell extracts. GST-Tir1 also coprecipitates purified forms of

alpha-actinin, talin, and vinculin while GST alone does not **bind** these three focal adhesion **proteins**. Biotinylated probes of these three **proteins** also bound Tir1 cleaved from GST. Similar associations of alpha-actinin, talin, and vinculin were also detected with the C-terminus of **Tir**, i.e., Tir3, the last 217 amino acids. Antibody staining of **EPEC**-infected cultured cells reveals the presence of focal adhesion **proteins** beneath the attached bacteria. Our experiments support a model in which the cytoplasmic domains of **Tir** recruit a number of focal adhesion **proteins** that can **bind** actin filaments to form pedestals. Since pedestals also contain villin, tropomyosin and myosin II [Sanger et al., 1996: Cell Motil. Cytoskeleton 34:279-287], the pedestals appear to be a novel structure sharing properties of both focal adhesions and microvilli.



L15 ANSWER 19 OF 43 MEDLINE DUPLICATE 14

ACCESSION NUMBER: 2000092453 MEDLINE
DOCUMENT NUMBER: 20092453 PubMed ID: 10628831
TITLE: Antibody response of patients infected with verocytotoxin-producing Escherichia coli to **protein** antigens encoded on the LEE locus.
AUTHOR: Jenkins C; Chart H; Smith H R; Hartland E L; Batchelor M; Delahay R M; Dougan G; Frankel G
CORPORATE SOURCE: Central Public Health Laboratory, London.
SOURCE: JOURNAL OF MEDICAL MICROBIOLOGY, (2000 Jan) 49 (1) 97-101.
Journal code: J2N; 0224131. ISSN: 0022-2615.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000124
Last Updated on STN: 20000124
Entered Medline: 20000113

AB Sera from patients infected with verocytotoxin-producing Escherichia coli (VTEC) 0157, from patients with antibodies to E. coli 0157 lipopolysaccharide (LPS) and from healthy controls were examined for antibodies to **proteins** involved in expressing the **attaching** and **effacing** phenotype. After SDS-PAGE, purified recombinant **intimin**, EspA-filament structural **protein**, translocated **protein** EspB and three separate domains of the **translocated intimin receptor** (**Tir**) were tested for reaction with patients' sera by immunoblotting. An ELISA was also used to detect antibodies to **intimin** in sera from E. coli 0157 LPS

antibody-positive individuals. Seven of nine culture-positive patients and one control patient had antibodies to EspA. Five of these patients and two controls had serum antibodies to the **intimin-binding** region of **Tir**, whereas none of the sera contained antibodies **binding** to either of the intracellular domains of **Tir**. By immunoblotting, 10 of 14 culture-positive patients had antibodies to the conserved region of **intimin**, eight of whom were infected with E. coli 0157 phage type 2. Thirty-six of 60 sera from culture-negative but E. coli 0157 LPS antibody-positive patients had antibodies to **intimin** as determined by ELISA. The secreted **proteins** are expressed in vivo during infection and are considered as pathogenic markers. Antibodies to these **proteins** may form the basis of a serodiagnostic test for the detection of patients infected with VTEC which carry the locus for the enterocyte effacement pathogenicity island and provide an adjunct test to the established serological tests based on VTEC LPS.

L15 ANSWER 20 OF 43 BIOSIS COPYRIGHT 2001 BIOSIS ' DUPLICATE 15
ACCESSION NUMBER: 2000:88974 BIOSIS
DOCUMENT NUMBER: PREV200000088974
TITLE: Antibody response of patients infected with verocytotoxin-producing Escherichia coli to **protein** antigens encoded on the LEE locus.
AUTHOR(S): Jenkins, C.; Chart, H. (1); Smith, H. R.; Hartland, E. L.; Batchelor, M.; Delahay, R. M.; Dougan, G.; Frankel, G.
CORPORATE SOURCE: (1) Laboratory of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London, NW9 5HT UK
SOURCE: Journal of Medical Microbiology, (Jan., 2000) Vol. 49, No. 1, pp. 91-101.
ISSN: 0022-2615.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Sera from patients infected with verocytotoxin-producing Escherichia coli (VTEC) O157, from patients with antibodies to E. coli O157 lipopolysaccharide (LPS) and from healthy controls were examined for antibodies to **proteins** involved in expressing the **attaching** and **effacing** phenotype. After SDS-PAGE, purified recombinant **intimin**, EspA-filament structural **protein**, translocated **protein** EspB and three separate domains of the **translocated intimin receptor** (**Tir**) were tested for reaction with patients' sera by immunoblotting. An ELISA was also used to detect antibodies to **intimin** in sera from E. coli O157 LPS antibody-positive individuals. Seven of nine culture-positive

patients and one control patient had antibodies to EspA. Five of these patients and two controls had serum antibodies to the **intimin-binding** region of **Tir**, whereas none of the sera contained antibodies **binding** to either of the intracellular domains of **Tir**. By immunoblotting, 10 of 14 culture-positive patients had antibodies to the conserved region of **intimin**, eight of whom were infected with E. coli O157 phage type 2. Thirty-six of 60 sera from culture-negative but E. coli O157 LPS antibody-positive patients had antibodies to **intimin** as determined by ELISA. The secreted **proteins** are expressed in vivo during infection and are considered as pathogenic markers. Antibodies to these **proteins** may form the basis of a serodiagnostic test for the detection of patients infected with VTEC which carry the locus for the enterocyte effacement pathogenicity island and provide an adjunct test to the established serological tests based on VTEC LPS.

L15 ANSWER 21 OF 43 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 2000094139 MEDLINE
DOCUMENT NUMBER: 20094139 PubMed ID: 10630443
TITLE: Human colostrum and serum contain antibodies reactive to the **intimin-binding** region of the **enteropathogenic** Escherichia **coli translocated intimin receptor**.
AUTHOR: Sanches M I; Keller R; Hartland E L; Figueiredo D M; Batchelor M; Martinez M B; Dougan G; Careiro-Sampaio M M; Frankel G; Trabulsi L R
CORPORATE SOURCE: Departamento de Microbiologia, Instituto de Ciencias Biomedicas, Sao Paulo, Brazil.
SOURCE: JOURNAL OF PEDIATRIC GASTROENTEROLOGY AND NUTRITION, (2000 Jan) 30 (1) 73-7.
Journal code: JL6; 8211545. ISSN: 0277-2116.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000204
Last Updated on STN: 20000204
Entered Medline: 20000121

AB BACKGROUND: In Brazil, **enteropathogenic** Escherichia **coli** (**EPEC**) diarrhoea is endemic in young infants. A characteristic feature of **EPEC** adhesion to host cells is intimate attachment leading to the formation of distinctive " **attaching** and **effacing**" (**A/E**) lesions on mammalian cells. Two genes directly involved in intimate adhesion, eae and **tir**, encode the adhesion molecule

**intimin** and its translocated receptor **Tir**, respectively. The **intimin-binding** domain of **Tir** was recently mapped to the middle part of the **polypeptide** (**Tir**-M), and the amino (**Tir**-N) and carboxy (**Tir**-C) termini were found to be located within infected host cells. Recently, it was shown that colostrum samples from mothers living in Sao Paulo contain IgA-class antibodies reactive with a number of **proteins** associated with **EPEC** virulence. It has also been shown that patients infected with verocytotoxin-producing E. coli O157 can produce antibodies to **Tir**. In the current study antibody responses to the different **Tir** domains were analyzed in sera and colostrum samples collected in an **EPEC**-endemic area of Brazil. METHODS: Recombinant **Tir**, **Tir**-N, **Tir**-M, and **Tir**-C were expressed as His-tagged **protein** in E. coli BL21a and purified on nickel columns. Western blot analysis was used to investigate colostrum IgA- and serum IgG-class antibodies reactive with the **Tir** fragments. RESULTS: Anti-**Tir** IgG antibodies were detected in the serum of children, with (63%) or without (50%) diarrhoea. Anti-**Tir** IgA-class antibodies were detected in all the colostrum pools tested. With the use of both serum IgG- and colostrum IgA-class antibodies, an immunodominant domain of the **Tir-polypeptide**, **Tir** M, was identified. CONCLUSION: The **intimin-binding** region of **Tir** (**Tir**-M) is the immunodominant region of the **polypeptide** in humans. Both serum IgG-class and colostrum IgA-class antibodies reacted predominantly with the **Tir**-M domain.

L15 ANSWER 22 OF 43 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2000:89908 BIOSIS
DOCUMENT NUMBER: PREV200000089908
TITLE: Human colostrum and serum contain antibodies reactive to the **intimin-binding** region of the **enteropathogenic** Escherichia **coli translocated intimin receptor**.
AUTHOR(S): Imperio Sanches, Marcela; Keller, Rogeria; Hartland, Elizabeth L.; Figueiredo, Dayse M. M.; Batchelor, Miranda; Martinez, Marina B.; Dougan, Gordon; Careiro-Sampaio, Magda M. S.; Frankel, Gad (1); Trabulsi, Luiz R.
CORPORATE SOURCE: (1) Department of Biochemistry, Imperial College, London, SW7 2AZ UK
SOURCE: JPGN, (Jan., 2000) Vol. 30, No. 1, pp. 73-77.
DOCUMENT TYPE: Article
LANGUAGE: English

SUMMARY LANGUAGE: English

AB Background: In Brazil, **enteropathogenic** Escherichia **coli** (**EPEC**) diarrhoea is endemic in young infants. A characteristic feature of **EPEC** adhesion to host cells is intimate attachment leading to the formation of distinctive " **attaching** and **effacing**" (**A/E**) lesions on mammalian cells. Two genes directly involved in intimate adhesion, eae and **tir**, encode the adhesion molecule **intimin** and its translocated receptor **Tir**, respectively. The **intimin-binding** domain of **Tir** was recently mapped to the middle part of the **polypeptide** (**Tir**-M), and the amino (**Tir** -N) and carboxy (**Tir**-C) termini were found to be located within infected host cells. Recently, it was shown that colostrum samples from mothers living in Sao Paulo contain IgA-class antibodies reactive with a number of **proteins** associated with **EPEC** virulence. It has also been shown that patients infected with verocytotoxin-producing E. coli O157 can produce antibodies to **Tir**. In the current study antibody responses to the different **Tir** domains were analyzed in sera and colostrum samples collected in an **EPEC**-endemic area of Brazil. Methods: Recombinant **Tir**, **Tir**-N, **Tir**-M, and **Tir**-C were expressed as His-tagged **protein** in E. coli BL21a and purified on nickel columns. Western blot analysis was used to investigate colostrum IgA- and serum IgG-class antibodies reactive with the **Tir** fragments. Results: Anti-**Tir** IgG antibodies were detected in the serum of children, with (63%) or without (50%) diarrhoea. Anti-**Tir** IgA-class antibodies were detected in all the colostrum pools tested. With the use of both serum IgG- and colostrum IgA-class antibodies, an immunodominant domain of the **Tir-polypeptide**, **Tir** M, was identified. Conclusion: The **intimin-binding** region of **Tir** (**Tir**-M) is the immunodominant region of the **polypeptide** in humans. Both serum IgG-class and colostrum IgA-class antibodies reacted predominantly with the **Tir**-M domain.

L15 ANSWER 23 OF 43 MEDLINE DUPLICATE 17

ACCESSION NUMBER: 2001181798 MEDLINE

DOCUMENT NUMBER: 21117269 PubMed ID: 11207558

TITLE: **Enteropathogenic** Escherichia **coli** (**EPEC**) attachment to epithelial cells: exploiting the host cell cytoskeleton from the outside.

AUTHOR: Celli J; Deng W; Finlay B B

CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, Canada.

SOURCE: CELLULAR MICROBIOLOGY, (2000 Feb) 2 (1) 1-9. Ref: 55
Journal code: DW3; 100883691. ISSN: 1462-5814.
PUB. COUNTRY: England: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010329

AB **Enteropathogenic** Escherichia **coli** (**EPEC**), a leading cause of human infantile diarrhoea, is the prototype for a family of intestinal bacterial pathogens that induce **attaching** and **effacing** (**A/E**) lesions on host cells. **A/E** lesions are characterized by localized effacement of the brush border of enterocytes, intimate bacterial attachment and pedestal formation beneath the adherent bacteria. As a result of some recent breakthrough discoveries, **EPEC** has now emerged as a fascinating paradigm for the study of host-pathogen interactions and cytoskeletal rearrangements that occur at the host cell membrane. **EPEC** uses a type III secretion machinery to attach to epithelial cells, translocating its own receptor for intimate attachment, **Tir**, into the host cell, which then **binds** to **intimin** on the bacterial surface. Studies of **EPEC**-induced cytoskeletal rearrangements have begun to provide clues as to the mechanisms used by this pathogen to subvert the host cell cytoskeleton and signalling pathways. These findings have unravelled new ways by which pathogenic bacteria exploit host processes from the cell surface and have shed new light on how **EPEC** might cause diarrhoea.

L15 ANSWER 24 OF 43 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-337712 [28] WPIDS
DOC. NO. NON-CPI: N1999-253081
DOC. NO. CPI: C1999-099316
TITLE: New **translocated intimin receptor** useful for treating infection by **enteropathogenic** or **enterohemorrhagic** Escherichia **coli**
DERWENT CLASS: B04 D16 S03
INVENTOR(S): DEVINNEY, R; FINLAY, B B; KENNY, B; STEIN, M
PATENT ASSIGNEE(S): (UYBR-N) UNIV BRITISH COLUMBIA
COUNTRY COUNT: 82
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|---|---|---|---|---|
| WO 9924576 | A1 | 19990520 | (199928)* | EN | 91 |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW | | | | | |
| W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW | | | | | |
| AU 9911373 | A | 19990531 | (199941) | | |
| EP 1029054 | A1 | 20000823 | (200041) | EN | |
| R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE | | | | | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---|---|---|---|
| WO 9924576 | A1 | WO 1998-CA1042 | 19981110 |
| AU 9911373 | A | AU 1999-11373 | 19981110 |
| EP 1029054 | A1 | EP 1998-954076 | 19981110 |
| | | WO 1998-CA1042 | 19981110 |

FILING DETAILS:

| PATENT NO | KIND | | PATENT NO |
|---|---|---|---|
| AU 9911373 | A | Based on | WO 9924576 |
| EP 1029054 | A1 | Based on | WO 9924576 |

PRIORITY APPLN. INFO: US 1997-65130 19971112

AN 1999-337712 [28] WPIDS

AB WO 9924576 A UPAB: 19990719

NOVELTY - A **translocated intimin receptor (Tir) polypeptide** that **binds intimin**, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated polynucleotide (I) encoding **Tir**;

(2) a polynucleotide selected from:

(a) the 1920 bp sequence (Ia) given in the specification;

(b) (Ia) where T is U;

(c) nucleic acid sequences complementary to (a) or (b);

(d) fragments of (a), (b) or (c) that are at least 15 nucleotides long and that hybridize to DNA which encode the 549 amino acid **polypeptide** defined in the specification;

(3) a polynucleotide selected from:

(a) the 1723 bp sequence (Ib) given in the specification;

(b) (Ib) where T is U;

(c) nucleic acid sequences complementary to (a) or (b);

(d) fragments of (a), (b) or (c) that are at least 15 nucleotides long and that hybridize to DNA which encode the 559 amino acid **polypeptide** defined in the specification;

(4) a vector containing (I);

(5) a host cell containing the vector of (4);

(6) an anti-**Tir** antibody;

(7) detecting **Tir** or its polynucleotides in a sample comprising:

(a) contacting the sample with an anti-**Tir** antibody or a nucleic acid probe that hybridizes to the **Tir** polynucleotide;

(b) detecting **binding** of the antibody to **Tir** **polypeptide**, where **binding** is indicative of the presence of the **Tir polypeptide** in the sample; or hybridization of the probe with the **Tir** polynucleotide which is indicative of **Tir** polynucleotide in the sample;

(8) a recombinant method for the production of **Tir** polynucleotides and **polypeptides**;

(9) a polynucleotide produced by (8);

(10) a host cell containing the polynucleotide of (9);

(11) production of a **Tir** fusion **protein**;

(12) identifying a compound that interferes with **binding** of **Tir** to **intimin** comprises comparing the **binding** of the **Tir** **polypeptide** to **intimin** in the presence and absence of the compound ;

(13) a method for differentiating among **attaching** and **effacing** pathogens by contacting them with an anti-**Tir** antibody and an anti-phosphotyrosine antibody;

(14) delivering a compound of interest to a **Tir** -containing cell by administering to the cell an **intimin** -containing cell delivery vehicle that contains a compound of interest;

(15) kits for detection of **Tir polypeptides** or polynucleotides; and

(16) a method for inducing a cell-mediated immune response to a **polypeptide** of interest, by contacting a subject with an attenuated bacteria, where the bacteria lacks an EspA or EspB **protein** and where the bacteria contains a polynucleotide encoding a **Tir** fusion **protein**.

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - Vaccine.

USE - Tir antibodies can be used to detect Tir in tissue or biological fluids, where presence of Tir is indicative of infection by enteropathogenic or enterohemorrhagic Escherichia coli (designated EPEC and EHEC, respectively). The antibody is able to

differentiate among attaching and effacing pathogens, when used in conjunction with an anti-phosphotyrosine antibody. Tir can be used to induce an immune response in humans or cows against EPEC or EHEC to ameliorate diseases caused by the Tir-producing EPEC or EHEC. Tir polynucleotides can be used as probes to detect the presence of Tir polynucleotides in a sample. Tir can also be used to detect a cell cytoskeleton. Additionally, Tir can be used to identify compounds that interfere with Tir binding to intimin. The Tir fusion proteins can be used in attenuated Escherichia coli to induce a cell-mediated immune response to polypeptides of interest, e.g. antigens (all Claimed).
Dwg.0/9

L15 ANSWER 25 OF 43 TOXLIT
ACCESSION NUMBER: 1999:23044 TOXLIT
DOCUMENT NUMBER: CA-130-333761K
TITLE: Pathogenic Escherichia coli **intimin** receptor **Tir** and gene **tir** and methods for detecting gene **tir** or **Tir protein** and for drug screening.
AUTHOR: Finlay BB; Kenny B; Devinney R; Stein M
SOURCE: (1999). PCT Int. Appl. PATENT NO. 9924576 05/20/1999 (University of British Columbia).
CODEN: PIXXD2.
PUB. COUNTRY: CANADA
DOCUMENT TYPE: Patent
FILE SEGMENT: CA
LANGUAGE: English
OTHER SOURCE: CA 130:333761
ENTRY MONTH: 199906
AB A **polypeptide**, called **Tir** (for **translocated intimin receptor**), which is secreted by **attaching** and **effacing** pathogens, such as the **enteropathogenic** (**EPEC**) and **enterohemorrhagic** (**EHEC**) E. **coli** is disclosed. These bacterial pathogens insert their own receptors into mammalian cell surfaces, to which the bacterial pathogen then adheres to trigger addnl. host signaling events and actin nucleation. Diagnosis of disease caused by pathogenic E. coli can be performed by the use of antibodies which **bind** to **Tir** to detect the **protein** or the use of nucleic acid probes for detection of nucleic acids encoding **Tir polypeptide**. Isolated nucleic acid sequences encoding **Tir polypeptide**, **Tir peptides**, a recombinant method for producing recombinant **Tir**, antibodies which **bind** to **Tir**, and a kit for the detection of **Tir**-producing E. coli are provided. A method of immunizing a host with **Tir** to induce a protective

immune response to **Tir** or a second **polypeptide** of interest is also provided. A method for screening for compds. which interfere with the **binding** of bacterial pathogens to their receptors is further provided. Thus, **protein Hp90**, previously believed to be a host membrane **protein**, has been identified as an **EHEC**- or **EPEC**-secreted **protein** which acts as an **intimin** receptor. **Proteins** encoded by the espA and espB genes were necessary for delivery of **Tir** to the host membrane.

L15 ANSWER 26 OF 43 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 1999242825 MEDLINE
DOCUMENT NUMBER: 99242825 PubMed ID: 10225900
TITLE: **Enterohemorrhagic** Escherichia **coli** O157:H7 produces **Tir**, which is translocated to the host cell membrane but is not tyrosine phosphorylated.
AUTHOR: DeVinney R; Stein M; Reinscheid D; Abe A; Ruschkowski S; Finlay B B
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada.
SOURCE: INFECTION AND IMMUNITY, (1999 May) 67 (5) 2389-98. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF125993
ENTRY MONTH: 199905
ENTRY DATE: Entered STN: 19990601
Last Updated on STN: 19990601
Entered Medline: 19990518

AB Intimate attachment to the host cell leading to the formation of **attaching** and **effacing** (**A/E**) lesions is an essential feature of **enterohemorrhagic** Escherichia **coli** (**EHEC**) O157:H7 pathogenesis. In a related pathogen, **enteropathogenic** E. **coli** (**EPEC**), this activity is dependent upon translocation of the **intimin** receptor, **Tir**, which becomes tyrosine phosphorylated within the host cell membrane. In contrast, the accumulation of tyrosine-phosphorylated **proteins** beneath adherent **EHEC** bacteria does not occur, leading to questions about whether **EHEC** uses a **Tir**-based mechanism for adherence and **A/E** lesion formation. In this report, we demonstrate that **EHEC** produces a functional **Tir** that is inserted into host cell

membranes, where it serves as an **intimin** receptor. However, unlike in **EPEC**, in **EHEC Tir** is not tyrosine phosphorylated yet plays a key role in both bacterial adherence to epithelial cells and pedestal formation. **EHEC**, but not **EPEC**, was unable to synthesize **Tir** in Luria-Bertani medium but was able to secrete **Tir** into M9 medium, suggesting that **Tir** synthesis and secretion may be regulated differently in these two pathogens. **EHEC Tir** and **EPEC Tir** both **bind intimin** and focus cytoskeletal rearrangements, indicating that tyrosine phosphorylation is not needed for pedestal formation. **EHEC** and **EPEC intimins** are functionally interchangeable, but **EHEC Tir** shows a much greater affinity for **EHEC intimin** than for **EPEC intimin**. These findings highlight some of the differences and similarities between **EHEC** and **EPEC** virulence mechanisms, which can be exploited to further define the molecular basis of pedestal formation.

L15 ANSWER 27 OF 43 MEDLINE DUPLICATE 19
ACCESSION NUMBER: 1999195823 MEDLINE
DOCUMENT NUMBER: 99195823 PubMed ID: 10096089
TITLE: Phosphorylation of tyrosine 474 of the **enteropathogenic** Escherichia **coli** (**EPEC**) **Tir** receptor molecule is essential for actin nucleating activity and is preceded by additional host modifications.
AUTHOR: Kenny B
CORPORATE SOURCE: Department of Pathology and Microbiology, School of Medical Sciences, Bristol, UK.. B.Kenny@bristol.ac.uk
SOURCE: MOLECULAR MICROBIOLOGY, (1999 Feb) 31 (4) 1229-41. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990727
Last Updated on STN: 19990727
Entered Medline: 19990715

AB The **enteropathogenic** Escherichia **coli** (**EPEC**) **Tir protein** becomes tyrosine phosphorylated in host cells and displays an increase in apparent molecular mass. The interaction of **Tir** with the **EPEC** outer membrane **protein**, **intimin**, triggers actin nucleation beneath the adherent bacteria. The **enterohaemorrhagic** E. **coli** 0157:H7 (**EHEC**

) **Tir** molecule is not tyrosine phosphorylated. In this paper, **Tir** tyrosine phosphorylation is shown to be essential for actin nucleation activity, but not for the increase in apparent molecular mass observed in target cells. Tyrosine phosphorylation had no role in **Tir** molecular mass shift, indicating additional host modifications. Analysis of **Tir** intermediates indicates that tyrosine-independent modification functions to direct **Tir**'s correct insertion from the cytoplasm into the host membrane. Deletion analysis identified **Tir** domains participating in translocation, association with the host membrane, modification and antibody recognition. **Intimin** was found to **bind** a 55-amino-acid region (TIBA) within **Tir** that topological and sequence analysis suggests is located in an extracellular loop. Homologous TIBA sequences exist in integrins, which also **bind** **intimin**. Collectively, this study provides definitive evidence for the importance of tyrosine phosphorylation for **EPEC Tir** function and reveals differences in the pathogenicity of **EPEC** and **EHEC**. The data also suggest a mechanism for **Tir** insertion into the host membrane, as well as providing clues to the mode of **intimin** -integrin interaction.

L15 ANSWER 28 OF 43 MEDLINE DUPLICATE 20
ACCESSION NUMBER: 1999440168 MEDLINE
DOCUMENT NUMBER: 99440168 PubMed ID: 10510232
TITLE: Identification of CesT, a chaperone for the type III secretion of **Tir** in **enteropathogenic** Escherichia **coli**.
AUTHOR: Elliott S J; Hutcheson S W; Dubois M S; Mellies J L; Wainwright L A; Batchelor M; Frankel G; Knutton S; Kaper J B
CORPORATE SOURCE: Center for Vaccine Development and Department of Microbiology and Immunology, University of Maryland School of Medicine, 685 W Baltimore St, Baltimore, MD 21201, USA.
CONTRACT NUMBER: AI21657 (NIAID)
AI41325 (NIAID)
SOURCE: MOLECULAR MICROBIOLOGY, (1999 Sep) 33 (6) 1176-89. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199911
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991104

AB The locus of enterocyte effacement of **enteropathogenic** Escherichia **coli** encodes a type III secretion system, an outer membrane **protein** adhesin (**intimin**, the product of eae ) and **Tir**, a translocated **protein** that becomes a host cell receptor for **intimin**. Many type III secreted **proteins** require chaperones, which function to stabilize **proteins**, prevent inappropriate **protein-protein** interactions and aid in secretion. An open reading frame located between **tir** and eae, previously named orfU, was predicted to encode a **protein** with partial similarity to the Yersinia SycH chaperone. We examined the potential of the orfU gene product to serve as a chaperone for **Tir**. The orfU gene encoded a 15 kDa cytoplasmic **protein** that specifically interacted with **Tir** as demonstrated by the yeast two-hybrid assay, column **binding** and coimmunoprecipitation experiments. An orfU mutant was defective in **attaching-effacing** lesion formation and **Tir** secretion, but was unaffected in expression of other virulence factors. OrfU appeared to stabilize **Tir** levels in the cytoplasm, but was not absolutely necessary for secretion of **Tir**. Based upon the physical similarities, phenotypic characteristics and the demonstrated interaction with **Tir**, orfU is redesignated as cesT for the chaperone for E. coli secretion of T ir.

L15 ANSWER 29 OF 43 MEDLINE DUPLICATE 21
ACCESSION NUMBER: 1999440167 MEDLINE
DOCUMENT NUMBER: 99440167 PubMed ID: 10510231
TITLE: **Enteropathogenic** Escherichia **coli** **translocated intimin** **receptor**, **Tir**, requires a specific chaperone for stable secretion.
AUTHOR: Abe A; de Grado M; Pfuetzner R A; Sanchez-Sanmartin C; Devinney R; Puente J L; Strynadka N C; Finlay B B
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, Canada V6T 1Z3.
SOURCE: MOLECULAR MICROBIOLOGY, (1999 Sep) 33 (6) 1162-75. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199911
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991104

AB **Enteropathogenic** Escherichia **coli** (**EPEC** ) secretes several Esps (E. coli-secreted **proteins**) that

are required for full virulence. Insertion of the bacterial **protein Tir** into the host epithelial cell membrane is facilitated by a type III secretion apparatus, and at least EspA and EspB are required for **Tir** translocation. An **EPEC** outer membrane **protein, intimin**, interacts with **Tir** on the host membrane to establish intimate attachment and formation of a pedestal-like structure. In this study, we identified a **Tir** chaperone, CesT, whose gene is located between **tir** and eae (which encodes **intimin**). A mutation in cesT abolished **Tir** secretion into culture supernatants and significantly decreased the amount of **Tir** in the bacterial cytoplasm. In contrast, this mutation did not affect the secretion of the Esp **proteins**. The level of **tir** mRNA was not affected by the cesT mutation, indicating that CesT acts at the post-transcriptional level. The cesT mutant could not induce host cytoskeletal rearrangements, and displayed the same phenotype as the **tir** mutant. Gel overlay and GST pulldown assays demonstrated that CesT specifically interacts with **Tir**, but not with other Esp **proteins**. Furthermore, by using a series of **Tir** deletion derivatives, we determined that the CesT **binding** domain is located within the first 100 amino-terminal residues of **Tir**, and that the pool of **Tir** in the bacterial cytoplasm was greatly reduced when this domain was disrupted. Interestingly, this domain was not sufficient for **Tir** secretion, and at least the first 200 residues of **Tir** were required for efficient secretion. Gel filtration studies showed that **Tir**-CesT forms a large multimeric complex. Collectively, these results indicate that CesT is a **Tir** chaperone that may act as an anti-degradation factor by specifically **binding** to its amino-terminus, forming a multimeric stabilized complex.

L15 ANSWER 30 OF 43 MEDLINE DUPLICATE 22
ACCESSION NUMBER: 2000063142 MEDLINE
DOCUMENT NUMBER: 20063142 PubMed ID: 10594820
TITLE: Hierarchy in the expression of the locus of enterocyte effacement genes of **enteropathogenic** Escherichia **coli**.
AUTHOR: Friedberg D; Umanski T; Fang Y; Rosenshine I
CORPORATE SOURCE: Departments of Molecular Genetics and Biotechnology, The Hebrew University, Faculty of Medicine, POB 12272, Jerusalem 91120, Israel.
SOURCE: MOLECULAR MICROBIOLOGY, (1999 Dec) 34 (5) 941-52. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000229
Last Updated on STN: 20000229
Entered Medline: 20000211

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) elicit changes in host cell morphology and cause actin rearrangement, a phenotype that has commonly been referred to as **attaching/effacing** (AE) lesions. The ability of **EPEC** to induce AE lesions is dependent upon a type III **protein** secretion/translocation system that is encoded by genes clustered in a 35.6 kb DNA segment, named the locus of enterocyte effacement (LEE). We used transcriptional fusions between the green fluorescent **protein** (gfp) reporter gene and LEE genes rorf2, orf3, orf5, escJ, escV and eae, together with immunoblot analysis with antibodies against **Tir**, **intimin**, EspB and EspF, to analyse the genetic regulation of the LEE. The expression of all these LEE genes was strictly dependent upon the presence of a functional integration host factor (IHF). IHF **binds** specifically upstream from the ler (orf1) promoter and appears to activate expression of ler, orf3, orf5 and rorf2 directly. The ler-encoded Ler **protein** was involved in activating the expression of escJ, escV, **tir**, eae, espB and espF. Expression of both IHF and Ler was needed to elicit actin rearrangement associated with AE lesions. In conclusion, IHF directly activates the expression of the ler and rorf2 transcriptional units, and Ler in turn mediates the expression of the other LEE genes.

L15 ANSWER 31 OF 43 MEDLINE DUPLICATE 23
ACCESSION NUMBER: 1999377127 MEDLINE
DOCUMENT NUMBER: 99377127 PubMed ID: 10447884
TITLE: A novel chromosomal locus of **enteropathogenic** Escherichia **coli** (**EPEC**), which encodes a bfpT-regulated chaperone-like **protein**, TrcA, involved in microcolony formation by **EPEC**.
AUTHOR: Tobe T; Tatsuno I; Katayama E; Wu C Y; Schoolnik G K; Sasakawa C
CORPORATE SOURCE: Department of Bacteriology, Institute of Medical Science, University ofTokyo 108-0071, Japan.
CONTRACT NUMBER: AI39521 (NIAID)
SOURCE: MOLECULAR MICROBIOLOGY, (1999 Aug) 33 (4) 741-52. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AB016764
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991214

AB The bfpTVW operon, also known as the per operon, of **enteropathogenic** Escherichia **coli** (**EPEC**) is required for the transcriptional activation of the bfp operon, which encodes the major subunit and assembly machinery of bundle-forming pili (BFP). An immobilized T7-tagged BfpT fusion **protein** that **binds** specifically to upstream promoter sequences of bfpA and eae was used to 'fish out' from a promoter library other **EPEC** chromosomal fragments that are bound by the BfpT **protein**. After screening for promoters exhibiting bfpTVW-dependent expression, one was identified that was positively regulated by bfpTVW and that is not present in the chromosomes of two non-virulent E. coli laboratory strains, DH5alpha and HB101. Further analysis of this positively regulated promoter in **EPEC** showed that it resided within a 4.9 kb sequence that is not present in E. coli K12. This locus, located downstream of the potB gene, was found to contain four open reading frames (ORFs): bfpTVW-activated promoter was localized upstream of ORF1. An ORF1 knockout mutant produced less of the BFP structural subunit (BfpA) and formed smaller than normal adherent microcolonies on cultured epithelial cells; however, this mutation did not affect bfp transcription. An ORF1-His6 fusion **protein** specifically bound the preprocessed and mature forms of the BfpA **protein** and thus appears to stabilize the former within the cytoplasmic compartment. ORF1 therefore is a newly isolated **EPEC** chromosomal gene that encodes a chaperone-like **protein** involved in the production of BFP. Hence, ORF1 was designated trcA (bfpT-regulated chaperone-like **protein** gene). The TrcA **protein** also specifically bound 39 kDa and **90** **kDa proteins** that are expressed by **EPEC** but not by E. coli K12. The **90** **kDa** **protein** was revealed to be **intimin**, a **protein** product of the eae gene, which is required for the **EPEC** **attaching/effacing** phenotype, suggesting a direct interaction of TrcA with **intimin** in the cytoplasmic compartment.

L15 ANSWER 32 OF 43 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 1999:566977 SCISEARCH
THE GENUINE ARTICLE: 219LV
TITLE: Role of bacterial **intimin** in colonic hyperplasia and inflammation
AUTHOR: Higgins L M (Reprint); Frankel G; Connerton I; Goncalves N S; Dougan G; MacDonald T T

CORPORATE SOURCE: ST BARTHOLOMEWS & ROYAL LONDON SCH MED & DENT, DEPT PAEDIAT GASTROENTEROL, LONDON EC1A 7BE, ENGLAND (Reprint); UNIV LONDON IMPERIAL COLL SCI TECHNOL & MED, DEPT BIOCHEM, LONDON SW7 2AZ, ENGLAND; UNIV NOTTINGHAM, SCH BIOL SCI, DIV FOOD SCI, LOUGHBOROUGH LE12 5RD, LEICS, ENGLAND
COUNTRY OF AUTHOR: ENGLAND
SOURCE: SCIENCE, (23 JUL 1999) Vol. 285, No. 5427, pp. 588-591.
Publisher: AMER ASSOC ADVANCEMENT SCIENCE, 1200 NEW YORK AVE, NW, WASHINGTON, DC 20005.
ISSN: 0036-8075.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS; LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 24
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) cells adhere to gut epithelial cells through **intimin** alpha: the ligand for a bacterially derived epithelial transmembrane **protein** called the **translocated intimin receptor**, Citrobacter rodentium colonizes the mouse colon in a similar fashion and uses a different **intimin**: **intimin** beta. **Intimin** alpha was found to costimulate submitogenic signals through the T cell receptor. Dead **intimin** beta(+) C. rodentium, **intimin** a-transfected C. rodentium or E. coli strain K12, and **EPEC** induced mucosal hyperplasia identical to that caused by C. rodentium live infection, as well as a massive T helper cell-type 1 immune response in the colonic mucosa, Mutation of cysteine-937 of **intimin** to alanine reduced costimulatory activity in vitro and prevented immunopathology in vivo. The mucosal changes elicited by C. rodentium were interferon-gamma-dependent. Immunopathology induced by **intimin** enables the bacteria to promote conditions that are favorable for increased microbial colonization.

L15 ANSWER 33 OF 43 MEDLINE DUPLICATE 24
ACCESSION NUMBER: 1999115516 MEDLINE
DOCUMENT NUMBER: 99115516 PubMed ID: 9916050
TITLE: **Enteropathogenic** Escherichia **coli** inhibits phagocytosis.
AUTHOR: Goosney D L; Celli J; Kenny B; Finlay B B
CORPORATE SOURCE: Biotechnology Laboratory and Departments of Microbiology & Immunology and of Biochemistry & Molecular Biology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada.
SOURCE: INFECTION AND IMMUNITY, (1999 Feb) 67 (2) 490-5.

Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 19990324
Entered Medline: 19990309

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) interacts with intestinal epithelial cells, activating host signaling pathways leading to cytoskeletal rearrangements and ultimately diarrhea. In this study, we demonstrate that **EPEC** interacts with the macrophage-like cell line J774A.1 to inhibit phagocytosis by these cells. Antiphagocytic activity was also observed in cultured RAW macrophage-like cells upon **EPEC** infection. The **EPEC** antiphagocytic phenotype was dependent on the type III secretion pathway of **EPEC** and its secreted **proteins**, including EspA, EspB, and EspD. **Intimin** and **Tir** mutants displayed intermediate antiphagocytic activity, suggesting that intimate attachment mediated by **intimin-Tir binding** may also play a role in antiphagocytosis. Tyrosine dephosphorylation of several host **proteins** was observed following infection with secretion-competent **EPEC** but not with secretion-deficient mutants. Dephosphorylation was detectable 120 min after infection with **EPEC**, directly correlating with the onset of the antiphagocytic phenotype. Inhibition of **protein** tyrosine phosphatases by pervanadate treatment increased the number of intracellular wild-type **EPEC** organisms to levels seen with secretion-deficient mutants, suggesting that dephosphorylation events are linked to the antiphagocytic phenotype. No tyrosine phosphatase activity was detected with the **EPEC**-secreted **proteins**, suggesting that **EPEC** induces antiphagocytosis via a different mechanism than Yersinia species. Taken together, the present findings demonstrate a novel function for **EPEC**-secreted **proteins** in triggering macrophage **protein** tyrosine dephosphorylation and inhibition of phagocytosis.

L15 ANSWER 34 OF 43 MEDLINE DUPLICATE 25
ACCESSION NUMBER: 1999215579 MEDLINE
DOCUMENT NUMBER: 99215579 PubMed ID: 10201396
TITLE: Structure of the cell-adhesion fragment of **intimin** from **enteropathogenic** Escherichia **coli**.
AUTHOR: Kelly G; Prasannan S; Daniell S; Fleming K; Frankel G; Dougan G; Connerton I; Matthews S

CORPORATE SOURCE: Department of Biochemistry and Centre for Structural Biology, Imperial College of Science, Technology and Medicine, South Kensington, London, UK.
SOURCE: NATURE STRUCTURAL BIOLOGY, (1999 Apr) 6 (4) 313-8.
Journal code: B98; 9421566. ISSN: 1072-8368.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1INM
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990511
Last Updated on STN: 19990511
Entered Medline: 19990423

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) induce gross cytoskeletal rearrangement within epithelial cells, immediately beneath the attached bacterium. The C-terminal 280 amino acid residues of **intimin** (Int280; 30.1 kDa), a bacterial cell-adhesion molecule, mediate the intimate bacterial host-cell interaction. Recently, interest in this process has been stimulated by the discovery that the bacterial **intimin** receptor **protein** (**Tir**) is translocated into the host cell membrane, phosphorylated, and after **binding** **intimin** triggers the intimate attachment. Using multidimensional nuclear magnetic resonance (NMR) and combining perdeuteration with site-specific protonation of methyl groups, we have determined the global fold of Int280. This represents one of the largest, non-oligomeric **protein** structures to be determined by NMR that has not been previously resolved by X-ray crystallography. Int280 comprises three domains; two immunoglobulin-like domains and a C-type lectin-like module, which define a new family of bacterial adhesion molecules. These findings also imply that carbohydrate recognition may be important in **intimin**-mediated cell adhesion.

L15 ANSWER 35 OF 43 MEDLINE DUPLICATE 26
ACCESSION NUMBER: 1999232514 MEDLINE
DOCUMENT NUMBER: 99232514 PubMed ID: 10216868
TITLE: **Binding** of **intimin** from **enteropathogenic** Escherichia **coli** to **Tir** and to host cells.
AUTHOR: Hartland E L; Batchelor M; Delahay R M; Hale C; Matthews S; Dougan G; Knutton S; Connerton I; Frankel G
CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, UK.
SOURCE: MOLECULAR MICROBIOLOGY, (1999 Apr) 32 (1) 151-8.
Journal code: MOM; 8712028. ISSN: 0950-382X.

PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990715
Last Updated on STN: 19990715
Entered Medline: 19990707

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) induce characteristic **attaching** and **effacing** (**A/E**) lesions on epithelial cells. This event is mediated, in part, by **binding** of the bacterial outer membrane **protein**, **intimin**, to a second **EPEC protein**, **Tir** (**translocated intimin receptor**), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study, we have localized the **intimin-binding** domain of **Tir** to a central 107-amino-acid region, designated **Tir**-M. We provide evidence that both the amino- and carboxy-termini of **Tir** are located within the host cell. In addition, using immunogold labelling electron microscopy, we have confirmed that **intimin** can **bind** independently to host cells even in the absence of **Tir**. This **Tir**-independent interaction and the ability of **EPEC** to induce **A/E** lesions requires an intact lectin-like module residing at the carboxy-terminus of the **intimin polypeptide**. Using the yeast two-hybrid system and gel overlays, we show that **intimin** can **bind** both **Tir** and **Tir**-M even when the lectin-like domain is disrupted. These data provide strong evidence that **intimin** interacts not only with **Tir** but also in a lectin-like manner with a host cell **intimin** receptor.

L15 ANSWER 36 OF 43 MEDLINE DUPLICATE 27
ACCESSION NUMBER: 2000010115 MEDLINE
DOCUMENT NUMBER: 20010115 PubMed ID: 10540286
TITLE: The **Tir-binding** region of **enterohaemorrhagic** Escherichia **coli** **intimin** is sufficient to trigger actin condensation after bacterial-induced host cell signalling.
AUTHOR: Liu H; Magoun L; Luperchio S; Schauer D B; Leong J M
CORPORATE SOURCE: Department of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA 01655, USA.
CONTRACT NUMBER: 1S10 RR05734-01 (NCRR)
ES07020 (NIEHS)

SOURCE: MOLECULAR MICROBIOLOGY, (1999 Oct) 34 (1) 67-81.
Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991217

AB **Enterohaemorrhagic** Escherichia **coli** (**EHEC**) has emerged as an important agent of diarrhoeal disease. Attachment to host cells, an essential step during intestinal colonization by **EHEC**, is associated with the formation of a highly organized cytoskeletal structure containing filamentous actin, termed an **attaching** and **effacing** (**A/E**) lesion, directly beneath bound bacteria. The outer membrane **protein intimin** is required for the formation of this structure, as is **Tir**, a bacterial **protein** that is translocated into the host cell and is thought to function as a receptor for **intimin**. To understand **intimin** function better, we fused **EHEC intimin** to a homologous **protein**, Yersinia pseudotuberculosis invasin, or to maltose-**binding protein**. The N-terminal 539 amino acids of **intimin** were sufficient to promote outer membrane localization of the C-terminus of invasin and, conversely, the N-terminal 489 amino acids of invasin were sufficient to promote the localization of the C-terminus of **intimin**. The C-terminal 181 residues of **intimin** were sufficient to **bind** mammalian cells that had been preinfected with an **enteropathogenic** E. **coli** strain that expresses **Tir** but not **intimin**. **Binding** of **intimin** derivatives to preinfected cells correlated with **binding** to recombinant **Tir protein**. Finally, the 181-residue minimal **Tir-binding** region of **intimin**, when purified and immobilized on latex beads, was sufficient to trigger **A/E** lesions on preinfected mammalian cells.

L15 ANSWER 37 OF 43 MEDLINE DUPLICATE 28
ACCESSION NUMBER: 2001151121 MEDLINE
DOCUMENT NUMBER: 21115123 PubMed ID: 11207537
TITLE: Identification of the **intimin-binding** domain of **Tir** of **enteropathogenic** Escherichia **coli**.
AUTHOR: de Grado M; Abe A; Gauthier A; Steele-Mortimer O; DeVinney R; Finlay B B

CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, Canada.
SOURCE: CELLULAR MICROBIOLOGY, (1999 Jul) 1 (1) 7-17.
Journal code: DW3; 100883691. ISSN: 1462-5814.
PUB. COUNTRY: England: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010315

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) attaches intimately to mammalian cells via a bacterial outer membrane adhesion molecule, **intimin**, and its receptor in the host cell membrane, **Tir**. **Tir** is a bacterial **protein** translocated into the host cell membrane and tyrosine phosphorylated after insertion. **Tir-intimin binding** induces organized actin polymerization beneath the adherent bacteria, resulting in the formation of pedestal-like structures. A series of **Tir** deletion derivatives were constructed to analyse which **Tir** domains are involved in **intimin binding**. We have localized the **intimin-binding** domain (IBD) of **Tir** using a yeast two-hybrid system and a gel-overlay approach to a region of 109 amino acids that is predicted to be exposed on the surface of the plasma membrane. A truncated **Tir protein** lacking this domain was translocated to the host cell membrane and tyrosine phosphorylated, but failed to **bind intimin** or to induce either actin polymerization or **Tir** accumulation beneath the bacteria. These results indicate that only a small region of **Tir** is needed to **bind intimin** and support the predicted topology for **Tir**, with both N- and C-terminal regions in the mammalian cell cytosol. They also confirm that **Tir-intimin** interactions are needed for cytoskeletal organization. We have also identified N-terminal regions involved in **Tir** stability and **Tir** secretion to the media.

L15 ANSWER 38 OF 43 MEDLINE DUPLICATE 29
ACCESSION NUMBER: 1999003184 MEDLINE
DOCUMENT NUMBER: 99003184 PubMed ID: 9784578
TITLE: **Translocated intimin receptors** (**Tir**) of Shiga-toxigenic Escherichia coli isolates belonging to serogroups O26, O111, and O157 react with sera from patients with hemolytic-uremic syndrome and exhibit marked sequence heterogeneity.

AUTHOR: Paton A W; Manning P A; Woodrow M C; Paton J C
CORPORATE SOURCE: Molecular Microbiology Unit, Women's and Children's Hospital, North Adelaide, South Australia 5006.. patonj@wch.sa.gov.au
SOURCE: INFECTION AND IMMUNITY, (1998 Nov) 66 (11) 5580-6. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF025311; GENBANK-AF070067; GENBANK-AF070068; GENBANK-AF070069
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981123

AB The capacity to form **attaching** and **effacing** (**A/E**) lesions on the surfaces of enterocytes is an important virulence trait of several enteric pathogens, including **enteropathogenic** Escherichia **coli** (**EPEC**) and Shiga-toxigenic E. coli (STEC). Formation of such lesions depends upon an interaction between a bacterial outer membrane **protein** (**intimin**) and a bacterially encoded receptor **protein** (**Tir**) which is exported from the bacterium and translocated into the host cell membrane. **Intimin**, **Tir**, and several other **proteins** necessary for generation of **A/E** lesions are encoded on a chromosomal pathogenicity island termed the locus for enterocyte effacement (LEE). Reports of sequence heterogeneity and antigenic variation in the region of **intimin** believed to be responsible for receptor **binding** raise the possibility that the receptor itself is also heterogeneous. We have examined this by cloning and sequencing **tir** genes from three different STEC strains belonging to serogroups O26, O111, and O157. The deduced amino acid sequences for the **Tir** homologues from these strains varied markedly, exhibiting only 65.4, 80.2, and 56.7% identity, respectively, to that recently reported for **EPEC Tir**. STEC **Tir** is also highly immunogenic in humans. Western blots of E. coli DH5alpha expressing the various STEC **tir** genes cloned in pBluescript [but not E. coli DH5alpha(pBluescript)] reacted strongly with convalescent sera from patients with hemolytic-uremic syndrome (HUS) caused by known LEE-positive STEC. Moreover, no reaction was seen when the various clone lysates were probed with serum from a patient with HUS caused by a LEE-negative STEC or with serum from a healthy individual. Covariation of exposed epitopes on both **intimin** and **Tir** may be a means whereby STEC avoid host immune responses without compromising adhesin-receptor interaction.

L15 ANSWER 39 OF 43 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1998:373002 BIOSIS
DOCUMENT NUMBER: PREV199800373002
TITLE: Type III **protein** secretion systems in bacterial pathogens of animals and plants.
AUTHOR(S): Hueck, Christoph J. (1)
CORPORATE SOURCE: (1) Biozentrum Univ. Wuerzburg, Am Hubland, 97074 Wuerzburg Germany
SOURCE: Microbiology and Molecular Biology Reviews, (June, 1998) Vol. 62, No. 2, pp. 379-433. ISSN: 1092-2172.
DOCUMENT TYPE: General Review
LANGUAGE: English

L15 ANSWER 40 OF 43 MEDLINE DUPLICATE 30
ACCESSION NUMBER: 97342718 MEDLINE
DOCUMENT NUMBER: 97342718 PubMed ID: 9199415
TITLE: **Intimin**-dependent **binding** of **enteropathogenic** Escherichia **coli** to host cells triggers novel signaling events, including tyrosine phosphorylation of phospholipase C-gamma1.
AUTHOR: Kenny B; Finlay B B
CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, Canada.. bkenny@unixg.ubc.ca
SOURCE: INFECTION AND IMMUNITY, (1997 Jul) 65 (7) 2528-36. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19970805
Last Updated on STN: 19970805
Entered Medline: 19970721

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) interactions with HeLa epithelial cells induced the tyrosine phosphorylation of a host **protein** of approximately 150 kDa, Hp150. Phosphorylation of this **protein** band was dependent on the interaction of the **EPEC protein** **intimin** with epithelial cell surfaces and was correlated with pedestal formation. Hp150 phosphorylation was specifically inhibited by the addition of cytochalasin D, an inhibitor of actin polymerization, although this appeared to be an indirect effect preventing interaction of **intimin** with its receptor, tyrosine-phosphorylated **Hp90**, and thus triggering Hp150 phosphorylation. This suggests the involvement of an actin-based

movement of membrane-bound tyrosine-phosphorylated **Hp90** to allow its interaction with **intimin**. Analysis of the tyrosine-phosphorylated Hp150 **protein** demonstrated that it is heterogeneous in composition, with phospholipase C-gamma1 (PLC-gamma1) being a minor component. Activation of PLC-gamma1 by tyrosine phosphorylation leads to inositol triphosphate and Ca2+ fluxes, events detected following **EPEC** infection. **EPEC** also induced tyrosine dephosphorylation of host **proteins**, including a 240-kDa host **protein** (Hp240), following **EPEC** infection. **Protein** dephosphorylation appears to be a signaling event which occurs independently of **intimin**. Inhibition of host tyrosine dephosphorylation events by the addition of the tyrosine phosphatase inhibitor sodium vanadate did not prevent actin accumulation beneath the adherent bacteria. We conclude that **EPEC** induces two sets of signaling events following infection. One set is dependent on **EPEC proteins** secreted by the type III secretion pathway (EspA and EspB) which induces **Hp90** tyrosine phosphorylation and dephosphorylation of host phosphotyrosine **proteins**. The second set, which is also dependent on the first signaling events, requires **intimin** interaction with its receptor, tyrosine-phosphorylated **Hp90** , to trigger Hp150 and PLC-gamma1 tyrosine phosphorylation as well as pedestal formation. Inhibition of pedestal formation by tyrosine kinase inhibitors indicates an important role for tyrosine phosphorylation events during **EPEC** subversion of host processes.

L15 ANSWER 41 OF 43 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 97367828 EMBASE
DOCUMENT NUMBER: 1997367828
TITLE: **Enteropathogenic** E. **coli** ( **EPEC**) transfers its receptor for intimate adherence into mammalian cells.
AUTHOR: Kenny B.; DeVinney R.; Stein M.; Reinscheid D.J.; Frey E.A.; Finlay B.B.
CORPORATE SOURCE: B.B. Finlay, Biotechnology Laboratory, Dept. of Biochemistry/Molec. Biology, University of British Columbia, Vancouver, BC V6T 1Z3, Canada
SOURCE: Cell, (1997) 91/4 (511-520).
Refs: 31
ISSN: 0092-8674 CODEN: CELLB5
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 001 Anatomy, Anthropology, Embryology and Histology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Enteropathogenic** E. **coli** (**EPEC**) belongs to a group of bacterial pathogens that induce epithelial cell actin rearrangements resulting in pedestal formation beneath adherent bacteria. This requires the secretion of specific virulence **proteins** needed for signal transduction and intimate adherence. **EPEC** interaction induces tyrosine phosphorylation of a **protein** in the host membrane, **Hp90**, which is the receptor for the **EPEC** outer membrane **protein**, **intimin**. **Hp90** - **intimin** interaction is essential for intimate attachment and pedestal formation. Here, we demonstrate that **Hp90** is actually a bacterial **protein** (**Tir**). Thus, this bacterial pathogen inserts its own receptor into mammalian cell surfaces, to which it then adheres to trigger additional host signaling events and actin nucleation. It is also tyrosine-phosphorylated upon transfer into the host cell.

L15 ANSWER 42 OF 43 MEDLINE DUPLICATE 31
ACCESSION NUMBER: 96256278 MEDLINE
DOCUMENT NUMBER: 96256278 PubMed ID: 8654358
TITLE: A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation.
AUTHOR: Rosenshine I; Ruschkowski S; Stein M; Reinscheid D J; Mills S D; Finlay B B
CORPORATE SOURCE: Department of Biotechnology and Molecular Genetics, The Hebrew University, Faculty of Medicine, Israel.
SOURCE: EMBO JOURNAL, (1996 Jun 3) 15 (11) 2613-24. Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960808
Last Updated on STN: 19970203
Entered Medline: 19960730

AB **Enteropathogenic** E. **coli** (**EPEC**) belongs to a group of bacterial pathogens that induce actin accumulation beneath adherent bacteria. We found that **EPEC** adherence to epithelial cells mediates the formation of fingerlike pseudopods (up to 10 microm) beneath bacteria. These actin-rich structures also contain tyrosine phosphorylated host **proteins** concentrated at the pseudopod tip beneath adherent **EPEC**. Intimate bacterial adherence (and pseudopod formation) occurred only after prior bacterial induction of tyrosine phosphorylation of an epithelial membrane **protein**, **Hp90**, which then associates directly with an **EPEC**

adhesin, **intimin**. These interactions lead to cytoskeletal nucleation and pseudopod formation. This is the first example of a bacterial pathogen that triggers signals in epithelial cells which activates receptor **binding** activity to a specific bacterial ligand and subsequent cytoskeletal rearrangement.

L15 ANSWER 43 OF 43 MEDLINE DUPLICATE 32
ACCESSION NUMBER: 96186500 MEDLINE
DOCUMENT NUMBER: 96186500 PubMed ID: 8641808
TITLE: Expression of **attaching/effacing** activity by **enteropathogenic** Escherichia **coli** depends on growth phase, temperature, and **protein** synthesis upon contact with epithelial cells.
AUTHOR: Rosenshine I; Ruschkowski S; Finlay B B
CORPORATE SOURCE: Department of Biotechnology and Molecular Genetics, Faculty of Medicine, The Hebrew University, Jerusalem, Israel.
SOURCE: INFECTION AND IMMUNITY, (1996 Mar) 64 (3) 966-73. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960726
Last Updated on STN: 19970203
Entered Medline: 19960716

AB **Enteropathogenic** Escherichia **coli** (**EPEC**) induces tyrosine phosphorylation of a **90-kDa protein** (**Hp90**) in infected epithelial cells. This in turn facilitates intimate **binding** of **EPEC** via the outer membrane **protein intimin**, effacement of host cell microvilli, cytoskeletal rearrangement, and bacterial uptake. This phenotype has been commonly referred to as **attaching/effacing** (**A/E**). The ability of **EPEC** to induce **A/E** lesions was dependent on bacterial growth phase and temperature. Early-logarithmic-phase **EPEC** grown at 37 degrees C elicits strong **A/E** activity within minutes after infection of HeLa epithelial cells. **EPEC** de novo **protein** syntheses during the first minutes of interaction with the host cell was required to elicit **A/E** lesions. However, once formed, bacterial viability was not needed to maintain **A/E** lesions. The type of growth media and partial O2 pressure level do not seem to affect the ability of **EPEC** to cause **A/E** lesions. These results indicates that the **A/E** activity of **EPEC**

is tightly regulated by environmental and host factors.

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Searcher : Shears 308-4994